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BLOOD CHOLESTEROL IN EXPERIMENTAL HYPO- AND HYPERTHYROIDISM (RABBIT)[1]

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From the Departments of Physiology and Medicine, University of Chicago

Received for publication June 30, 1932

Definite changes in blood cholesterol in both clinical and experimental hypo- and hyperthyroidism have recently been reported. Leopo'd and Seisser (1923) reported a decrease in blood cholesterol when rabbits were fed thyroid extract. Hecksner (1925) reported that human cretins generally have an elevated blood cholesterol. Kunde (1926) reported that the blood of cretin rabbits shows a marked increase in cholesterol, which is decreased when thyroid is fed. Werner (1928) reported that thyroidectomy in guinea pigs, rabbits and sheep is followed by a marked increase in blood cholesterol. Laroche (1929) reported that in clinical cases of hyperthyroidism the blood cholesterol is low but becomes normal, parallel with a return to normal of the basal metabolic rate. Mason, Hunt and Hurxthal (1930) also reported decreased blood cholesterol in a long series of cases of hyperthyroidism, and decreased blood cholesterol in myxedematous patients.

The experiments reported in this paper were carried out to see if the changes in blood cholesterol of experimental hypo- and hyperthyroidism can be correlated with the changes in erythrocyte and Hb content that occur in these conditions.

METHODS. The literature on the technique of producing cretin and hyperthyroid rabbits has been previously cited (Kunde et al., 1927; Kunde and Proud, 1929). Hb is expressed in per cent, and was determined by the Newcomer method (1923). Cholesterol was determined on whole blood by Sackett's (1925) modification of Bloor's method. All determinations were made in duplicate.

RESULTS AND DISCUSSION. The results of the first experiment, in which

[1] This investigation was aided by grants from the Henry Strong Dennison Foundation, and from the Seymour Coman Fund.

three cretins and six normal animals were used, are tabulated in tables 1 and 2. Table 1 is a tabulation of the weight; r.b.c.s per cmm., Hb in grams per 100 cc. blood, and cholesterol in milligrams per 1000 cc. whole blood before and after thyroid feeding. The desiccated thyroid was fed for either two or four weeks.

TABLE 1

Series I. Effect of thyroid feeding on blood cholesterol

RABBIT	SEX	BEFORE THYROID FEEDING				THYROID DAILY	AFTER THYROID FEEDING				
		Weight	R.B.C. per mm³.	Hb	Cholesterol		Total amount fed	Weight	R.B.C. per mm³.	Hb	Cholesterol
		grams		*grams*	*mgm. per cent*	*grams*		*grams*		*grams*	*mgm. per cent*
Cretin, R. S.	♂	2,070	5,550,000	11.1	133.3	0.318	4.45	1,980	4,110,000	9.5	53.5
Cretin, L. H.	♀	2,120	3,930,000	9.2	125	0.318	4.45	1,860	4,070,000	10.6	78.0
Cretin, W.	♀	2,410	5,690,000	9.3	111.1	0.318	8.90	1,835	5,000,000	12.5	100.0
Cretin, O. C.	♂	1,867	5,930,000	12.4	90.9	0.318	Died, hyperthyroidism 10/2/31				
Normal, F.	♂	2,830	5,930,000	11.3	117.4	0.590	8.20	2,440	6,020,000	12.7	70.2
Normal, F. T.	♀	3,064	6,720,000	12.3	120.5	0.590	16.40	2,940	5,370,000	12.6	70.4
Normal, R. H.	♂	2,864	7,630,000	13.2	61.9	0.590	16.40	2,655	6,260,000	12.6	51.7
Normal, N. 12	♀	2,805	6,550,000	12.8	67.5	0.590	16.40	2,860	6,800,000	13.1	49.5
Normal, Fos.	♂	2,765	6,540,000	12.1	74.4	0.590	8.20	2,718	6,920,000	13.7	45.3
Normal, N. 12	♂	3,530	6,530,000	11.8	88.8	0.590	16.40	2,840	7,220,000	12.3	50.8

TABLE 2

Series I. Effect of thyroid feeding on blood cholesterol

ANIMAL	TOTAL AMOUNT OF THYROID FED	PER CENT CHANGES AFTER THYROID FEEDING				
		Weight	R. b. c.	Hb	Cholesterol	Period of feeding
	grams					*days*
Cretin, R. S., ♂	4.45	−4.3	−25.2	−13.9	−59.9	14
Cretin, L. H., ♀	4.45	−12.3	+3.5	+15.2	−37.6	14
Cretin, W., ♀	8.90	−23.8	−12.1	+34.4	−10.0	28
Cretin, O. G., ♂						
Normal, F., ♂	8.20	−10.2	+1.5	+12.4	−40.9	14
Normal, F. T, ♀	16.40	−4.0	−20.1	+2.4	−41.7	14
Normal, N. 12, ♀	16.40	+1.9	+3.8	+2.3	−26.6	14
Normal, Fos., ♂	16.40	−1.6	+5.8	+13.2	−39.1	28
Normal, R. H., ♂	8.20	−7.3	−17.9	−4.54	−7.2	28
Normal, N. 12, ♂	16.40	−19.5	+10.5	+4.23	−42.8	28

Thyroid feeding in both cretin and normal animals produced a decrease in blood cholesterol. When this decrease in blood cholesterol is compared with the corresponding change in Hb and r.b.c.s, however, there seems to be no constant correlation. In some cases, both r.b.c.s and Hb are increased, in other cases both are decreased, in others one may be decreased

and the other increased. Some normal rabbits show no significant change in red blood cells and Hb after thyroid feeding.

These results seemed to indicate that although thyroid feeding to both normal and cretin rabbits produces a decrease in whole blood cholesterol, this change is not correlated with a constant change in red blood cells and Hb.

TABLE 3

Series II

	ANIMAL					
	Cretin, N 12	Cretin, N 13	Normal, F.	Normal, F. T.	Normal, N 12	Normal, R. H.
Sex	♂	♀	♂	♀	♀	♂
Before thyroid feeding						
Preliminary determinations	11/18/31	11/18/31	11/18/31	11/18/31	11/18/31	11/18/31
Weight, grams	2,045	2,390	3,220	3,040	2,840	2,749
R. B. C., mm³	4,930,000	5,000,000	6,370,000	7,940,000	5,250,000	6,710,000
Hb, grams	12.5	13.1	10.0	14.0	10.1	13.6
Cholesterol, mgm. per cent	222.2	227.1	74.5	94.4	73.6	93.6
After thyroid feeding						
Determinations made	12/16/31	12/16/31	12/16/31	12/16/31	12/16/31	12/16/31
Weight, grams	1,490	1,695	2,460	2,080	2,230	2,060
R. B. C., mm³	5,100,000	7,840,000	6,450,000	5,850,000	5,190,000	7,780,000
Hb., grams	11.5	15.8	11.5	12.0	11.0	15.0
Cholesterol, mgm. per cent	84.0	164.8	83.2	99.0	86.4	86.8
Total thyroid fed, grams	8.04	8.04	12.5	12.5	12.5	12.5
Percentage changes						
Weight	−27.1	−28.6	−23.6	−31.2	−21.1	−24.7
R. B. C.	+3.4	+56.8	+1.2	−26.1	−1.1	+15.9
Hb	−8.0	+20.6	+15.0	−14.2	+7.8	+10.3
Cholesterol	−62.2	−27.4	+11.6	+4.8	+17.3	−7.2

A second experiment was run on a series of four normal animals (which were used in the previous experiment but allowed to recover from the effects of thyroid feeding), and two additional cretins (see table 3). All these animals were fed 0.238 gram (cretins), or 0.446 gram (normal), of desiccated thyroid daily for four weeks.

Both cretins show the same weight loss and severe symptoms of induced hyperthyroidism. In one case, however, there is no significant change in

red blood cells and Hb, but a 62.2 per cent decrease in cholesterol. The other shows marked increases in red blood cells and Hb with a 27.4 per cent decrease in cholesterol. The normal animals in this series show slight increases in blood cholesterol in three cases, and a slight decrease in a fourth case. These changes are not marked, but seem at least to indicate that the ingestion of desiccated thyroid in normal rabbits does not always cause an appreciable decrease in blood cholesterol. The variations in Hb and red blood cells cannot be correlated with changes in blood cholesterol.

CONCLUSIONS

1. The blood cholesterol in cretin rabbits is greater than in normal rabbits.

2. Feeding desiccated thyroid to cretin rabbits causes a reduction in the blood cholesterol. This may not always be apparent following thyroid feeding to normal rabbits.

3. The changes in blood cholesterol following the ingestion of desiccated thyroids in normal or cretin rabbits cannot be correlated with changes in red blood cells and Hb.

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THE RELATION OF INSULIN TO LIVER GLYCOGEN[1]

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Experimental work on the relationship of insulin to liver glycogen has shown that in depancreatized animals the liver becomes glycogen free in about 48 to 72 hours after pancreatectomy (Hinselmann, 1905; Fisher-Lackey, 1925; Macleod, 1926) and when insulin and sugar are given, glycogen storage occurs after 24 to 48 hours (Banting, Best, Collip and Macleod, 1922). On the other hand in the normal animal, against expectation, practically all investigators (McCormick and Macleod, 1923; Dudley and Marrian, 1923; Babkin, 1923; Brugsch, 1924; Nitzescu, 1924, and Gigon and Staub, 1924), have found that insulin given with or without sugar causes a decrease in liver glycogen or at least a retardation in glycogen formation. The exception to this is the work of Bissinger, Lesser and Zipf (1923), who have found that when insulin was injected along with glucose, three times as much glycogen was formed in the liver as when sugar was injected alone. But this action was only temporary and in about an hour the glycogen content of the liver returned to the hunger value.

In the experiments which one of us (R. C. Bodo) performed in collaboration with H. P. Marks (1928) on the isolated perfused mammalian liver, where a glycogen storage was obtained when the perfusing blood contained an abundant supply of dextrose, a glycogen breakdown also occurred when insulin was given.

From the experimental work it is not clear 1, whether the breakdown of liver glycogen observed in normal animals is a direct action of insulin on the liver or an indirect one, i.e., associated with the hypoglycemia caused by insulin; 2, whether the storage of glycogen in the liver of the depancreatized animal after insulin occurs gradually from the time of the administration of insulin or only after a delay (latent period). In the experiments performed by Banting, Best, Collip and Macleod (1922) on diabetic

[1] This paper was read at the annual meeting of the Federation of American Societies for Experimental Biology in Montreal, April, 1931. Abstract appeared in Journ. Biol. Chem., 1931, xcii, Proc. xxv.

animals, in which glycogen was found in the liver 24 hours following the administration of insulin, it is quite possible that the liver was glycogen-free in the first 18 hours and the storage occurred only in the last 6 hours. It is quite conceivable that insulin in the beginning would change the metabolism in such a manner that the liver would regain its glycogen storing ability and insulin would have no direct storing effect on the liver at all.

In the present paper we attempt to answer these two questions.

Methods. The experiments were carried out on dogs which had been fed on a high carbohydrate diet. Morphine and chloralose were used for anesthesia and in a few experiments morphine and amytal. Samples of liver (about 5 grams each) were cut out at hourly intervals, each time from a different lobe, bleeding being stopped by mass ligature. The liver samples were immediately transferred to 100 cc. pyrex centrifuge tubes which contained 7 cc. 60 per cent KOH solution and were fitted with a reflux condensor. The centrifuge tubes with the potash solution and reflux condensor were weighed beforehand and placed in a boiling water bath for 5 minutes before the liver sample was taken. The liver sample having been plunged in the hot potash, the centrifuge tube was replaced on the waterbath for 30 minutes, during which time the sample entirely dissolved. It was then cooled and weighed. The weight of the liver sample was determined by difference. For the glycogen determination a modified Pflüger's method was used. After 3 hours of potash hydrolysis in the waterbath, as much 95 per cent alcohol was used for precipitation as gave a final alcoholic concentration of 66 per cent. The tubes were allowed to stand 12 hours and then centrifuged. The precipitate was washed 3 times with alcohol, which was saturated with NaCl, and the tubes were again centrifuged. After the removal of the supernatant fluid, the precipitate was taken up in 20 cc. H_2O, first neutralized and then made up with H_2O and concentrated HCl to a final volume of 50 cc., the HCl content of the solution being 2.2 per cent, and then hydrolyzed for 3 hours on the water bath. The solution was next carefully neutralized and the sugar content determined by the Hagedorn-Jensen method.

Blood samples were taken for sugar analysis at times corresponding to the liver section, with the exception of the two first samples. The first blood sample was taken before morphine was injected and the second just before the chloralose was administered. For the blood sugar determinations, Benedict's colorimetric and the Hagedorn-Jensen methods were used.

Where dextrose was given, a slow continuous infusion was made into the portal vein, hepatic artery, femoral vein or jugular vein. With the portal infusion, the cannula was inserted into the splenic root, and with the hepatic artery infusion, the cannula was placed into the gastroduodenal artery and the solution infused backwards under 150 mm. pressure. For

each kilogram body weight 0.80 gram of dextrose, dissolved in 120 cc. H_2O, was infused hourly. According to Woodyatt (1916) this is the maximum amount of dextrose that can be given without causing glycosuria. Liver samples were taken also for the determination of the water content.

Where insulin, commercial or crystalline,[2] was given, this was injected intravenously or subcutaneously either in single doses, or infused at a slow constant rate into the portal or jugular vein.

The experiments usually lasted 5 to 7 hours from the beginning of the sugar infusion. Throughout the whole of the experiment the animal was kept warm, rectal temperature being taken regularly, the lungs were ventilated by means of Starling's Ideal respiration pump (manufactured by Palmer, London, England), and the blood pressure measured in the femoral artery with a mercurial manometer.

TABLE 1

Glycogen content of different liver lobes. Morphine-chloralose anesthesia

		TIME (HOURS)				
		0	3	4	5	
		per cent	*per cent*	*per cent*	*per cent*	
Dog 1	1. lobe	3.84	0.15	0.10		No sugar infusion
	2. lobe	3.86	0.16	0.10		
Dog 2	1. lobe				1.14	Sugar infusion
	2. lobe				1.25	
Dog 3	1. lobe				2.60	
	2. lobe				2.97	

A. *Effect of experimental technic on liver glycogen.* It occurred to us that dogs would be most suitable for an investigation of glycogen formation and breakdown, because we would be able to follow the changes in liver glycogen in the same animal, and large enough samples could be easily obtained, provided however, that an anesthetic could be found that would not prevent glycogen formation from glucose and also provided that the distribution of glycogen in the entire liver would remain uniform throughout.

In the first series of experiments, which we consider the basis for the present and following papers, we attempt to show that the technic employed does not affect materially the normal function of the liver in regard to glycogen.

In experiment 1 (table 2) it is shown that in a normal dog under morphine-chloralose anesthesia with no sugar infusion there occurs a steady

[2] We take this opportunity to express our indebtedness to Dr. E. M. K. Geiling for having supplied us with crystalline insulin.

decrease in liver glycogen during the five-hour period, the blood sugar falling slightly. On the other hand, if sugar is infused (expts. 2 and 3, table 2) a rise in liver glycogen occurs. In these experiments for every kilogram body weight 3 mgm. morphine sulphate were injected subcutaneously and 0.1 gram of chloralose intravenously. It was shown by Ross (1918), confirmed by Stewart and Rogoff (1922), and later by Pierce and Plant (1928), that morphine produces a hyperglycemia which is very different in degree in different animals, lasts for a few hours and reaches its maximum in a relatively short time. We have confirmed their statements, and though morphine may cause some breakdown in liver glycogen, we could not dispense with it, because it is well known that chloralose, if given alone, stimulates the spinal cord and produces convulsive move-

TABLE 2

Morphine (↑ M)-chloralose (↑ C). At ↑ D dextrose infusion started in experiment 2 into hepatic artery, in experiment 3 into portal vein. Rate: 120 cc. 10 per cent dextrose solution per hour. At ↑ M2 30 mgm. morphine sulphate injected intravenously. In experiment 1 no sugar infusion.

	TIME (HOURS)								
	0	½	1	2	3	4	5	6	7
Expt. 1 (Dog 12.7 kgm.):									
Blood sugar, mgm. per cent..			77	68	72	68	60	62	
Liver glycogen, per cent.....	↑ M	↑ C	4.86	4.09	3.42	3.06	3.10	2.39	
Expt. 2 (Dog 17.0 kgm.):									
Blood sugar, mgm. per cent..			108	225	206	127	97	110	
Liver glycogen, per cent.....	↑ M	↑ C	1.20 ↑ D	1.34	1.39	1.60	1.80	2.20	
Expt. 3 (Dog 18.0 kgm.):									
Blood sugar, mgm. per cent..	74	158			237	267	320	311	310
Liver glycogen, per cent.....	↑ M	↑ C	6.82 ↑ D		8.28 ↑ M2	7.23	7.10	8.66	7.66

ments which do not occur if small doses of morphine are given previously. From the rise in blood sugar observed in most of our experiments following the administration of morphine, we have to conclude that there must have been an initial, perhaps slight breakdown in liver glycogen, but that this did not affect the glycogen storing ability of the liver in the later stages of our experiments. In experiment 3 a second dose of morphine sulphate was given intravenously after the 2 hour infusion, because the animal became restless and this very probably caused the corresponding slight fall in the liver glycogen. In later experiments, if it became necessary to quiet the animal, a second small dose of chloralose was given without any appreciable effect on the liver glycogen.

In some experiments instead of chloralose, amytal (40 mgm. per kgm.) was given and as seen in experiment 4 (table 3) there was practically no change in liver glycogen; in experiment 5 (table 3) there was a distinct rise

in the first 3 hours and then a slight fall. As a whole, morphine and chloralose gave more satisfactory results and therefore these were the anesthetics chosen in the later experiments.

In all these experiments the maintenance of artificial respiration led to a better oxygenation of the blood and in some instances, when required, enabled us to further expose the liver by making a slight opening in the chest. Care was taken not to overventilate the animal, the rate of respiration being 12 to 14 per minute and the stroke volume adjusted according to the size of the animal. No alkalosis occurred in the experiments and the lactic acid content of the blood remained constant.

As regards the distribution of glycogen in the liver it has been shown by Macleod and Pierce (1911) and Noble and Macleod (1923), that it is fairly uniformly distributed in vivo and becomes irregular post mortem. In morphine-chloralose anesthesia with or without sugar infusion it was found

TABLE 3

Morphine (↑ M)-Amytal (↑ A). At ↑ D dextrose infusion into jugular vein started. Rate: 120 cc. 10 per cent solution per hour.

	TIME (HOURS)								
	0	½	1	2½	4		5½	7	8
Expt. 4 (Dog 15.2 kgm.):									
Blood sugar.....	95	89		133	163		153	167	175
Liver glycogen...	↑ M	↑ A600 mg.	5.13 ↑ D	4 97	5 04		4 62	4 60	4 76
	0	½	1	2	3		4	5	6
Expt. 5 (Dog 11.2 kgm.):									
Blood sugar.....	88	128		236	190		208	336	286
Liver glycogen...	↑ M	↑ A 300 mg.	1 24 ↑ D	1 69	1 92	↑ A 100 mg.	2 14	1 63	1 68

to be also uniform (table 1), making it possible to take a sample every hour from different lobes. In the earlier work on the isolated perfused liver it was stated that the different lobes contain different percentages of glycogen Since that time one of us (R. C. Bodo) in collaboration with V. Vendeg working on isolated livers improved the technic of perfusion and found that even in this preparation the distribution of glycogen is fairly uniform (unpublished paper). When an uneven distribution of glycogen occurred in the isolated liver, it was due very probably to a mechanical hindrance of the blood flow, caused by the falling back of one lobe. In other instances and in whole animals some toxic factors such as lactic acid, alkalosis, etc., were responsible.

The operative procedure, i.e., the laparotomy, the cutting of the liver and the application of mass ligature did not interfere with the glycogen storage, provided that the viscera were protected from cooling and handled gently.

During the usual five hour experiments the dextrose was infused in 600 cc. of water, and this amount of fluid did not change the water content of the liver.

From the above experiments, we conclude, that the morphine-chloralose anesthesia, the operative procedure employed, and the artificial respiration do not materially interfere with glycogen storage in the liver and its uniform distribution.

B. *Effect of insulin on liver glycogen in normal animals.* In the next group of experiments the effect of insulin on liver glycogen in normal animals was studied. In order to see whether the breakdown of liver glycogen observed by previous investigators in normal animals after the administration of insulin is caused by the hypoglycemia, a simultaneous and continuous sugar infusion was made. In experiments 6, 7 and 8 (table 4) the dextrose solution was infused into the splenic root of the portal vein, so that if the insulin would lower the blood sugar level in the general system, the liver cells would still be bathed with a hyperglycemic blood and tissue fluid. The hypoglycemic assumption was not substantiated by the experiments, since the infused sugar passed quickly through the liver and the blood sugar level never fell below normal. The same results were obtained when the sugar was infused into the hepatic artery and in the later experiments when the sugar was infused into the jugular or femoral vein. The insulin, in large doses, was injected slowly, taking 5 to 10 minutes for each injection, into the jugular veins. Crystalline insulin was used in experiments 6 and 7 and commercial insulin in experiment 8. In every instance a decrease in liver glycogen was obtained. Since in these experiments there was no hypoglycemia, one would conclude that the breakdown of liver glycogen in normal animals after insulin is not a secondary effect caused by the hypoglycemia, but is a direct effect of insulin on the liver. However, there may be an objection that though the blood sugar never fell below normal value in these experiments, the drop in the blood sugar curves, a "relative hypoglycemia," seen in experiments 6 and 7, could be sufficient to call upon the liver glycogen stores. But the decrease in liver glycogen obtained in experiment 8 with a steady rise in the blood sugar curve, produced by infusing increasing amounts of dextrose, obviates this objection.

If small, but effective amounts of insulin were continuously infused, the results were essentially the same. It was found that 4 units of insulin (dissolved in 10 cc. saline) per hour infused continuously, either into the portal or jugular vein, caused a marked fall in blood sugar (expt. 9, table 5). When 4 units of insulin were infused hourly with the sugar infusion as in experiments 10 and 11 (table 5), the liver glycogen remained practically constant throughout the entire experiment. The glycogen storage, which would occur with sugar alone, was retarded by the small amounts of insulin

TABLE 4

Morphine (↑M)-chloralose (↑C). At ↑D dextrose infusion into portal vein started. Rate: 120 cc./hour of 8 per cent dextrose solution in experiment 6, of 10 per cent solution in experiment 7. In experiment 8 rate at beginning 120 cc. 9 per cent dextrose solution/hour, this progressively increased. At ↑insulin injection into jugular vein (cryst. in expts. 6 and 7 and insulin (Lilly) in expt. 8).

	TIME (HOURS)							
	0	½	1	2	3	4	5	6
Expt. 6 (Dog 12.0 kgm.):								
Blood sugar....	78		156	112	100	141	235	258
Liver glycogen.	↑M	↑C 4.82 ↑D		4.24	4.39	4.21	3.81	3.58
			↑11	↑2.2	↑2.2 ↑2.2	↑2.2 ↑4.4	↑44 ↑4.4	total = 72.6 units
Expt. 7 (Dog 15.0 kgm.):								
Blood sugar....	90	94		125	110	192	275	415
Liver glycogen.	↑M	↑C	3.35 ↑D	2.90	2.17	2.01	0.93	1.17
			↑20 ↑7	↑7	↑7 ↑7	↑7 ↑7	↑7 ↑7	total = 76 units
Expt. 8 (Dog 14.2 kgm.):								
Blood sugar....	94	105		132	146	161	284	
Liver glycogen.	↑M	↑C	3.55 ↑D	2.88	2.90	2.73	2.11	
			↑20		↑20			total = 40 units

given. Experiment 10 shows a break in the blood sugar curve; in experiment 11 this is again prevented by the infusion of increasing amounts of sugar. The result as regards liver glycogen is in both cases the same.

From the experimental evidence presented, it follows that insulin in large doses causes a breakdown of liver glycogen, in small doses it prevents a storage in spite of a steadily increasing hyperglycemia. Therefore the breakdown cannot be due to hypoglycemia. The breakdown also obtained in previous experiments on the isolated perfused liver (Bodo and Marks, 1928), leads us to the conclusion that the breakdown is due to a direct liver action.

It is claimed by some investigators that insulin has an inhibiting effect on the glycogenolysis in the liver. Bornstein and Griesbach (1924), and later Issekutz (1927) reported experiments on perfused isolated liver, in which

TABLE 5

Morphine (↑M)-chloralose (↑C). At ↑D dextrose, at ↑I insulin intravenous infusion started. Rate: 120 cc. 10 per cent dextrose solution per hour, in experiment 11 progressively increased. Insulin (Lilly) 4 units in 10 cc. saline per hour.

	TIME (HOURS)							
	0	½	1	1½	2	2½	3	3½
Expt. 9 (Dog 24.0 kgm.):								
Blood sugar	76 ↑M	138 ↑C		111 ↑I	70	58	57	44
	0	½	1	2	3	4	5	6
Expt. 10 (Dog 13.0 kgm.):								
Blood sugar	112	133		100	128	176	245	253
Liver glycogen	↑M	↑C	2.78 ↑D, I	3.14	3.24	2.81	2.97	2.79
Expt. 11 (Dog 15.2 kgm.):								
Blood sugar	99	145		167		170	180	190
Liver glycogen	↑M	↑C	4.04 ↑D, I	4.52		5.07	4.75	3.99

they have found that, when insulin was given before adrenalin, the sugar production was not as marked as when adrenalin was given alone. This statement could not be confirmed by Bodo and Marks (1928), who found that the glycogenolysis caused by adrenalin was not influenced by insulin. More recently Sahyun and Luck (1929), and Cori, Cori and Buchwald (1930) claimed again that the decrease in liver glycogen, which occurs regularly at certain time intervals after the injection of adrenalin, can be entirely prevented by insulin.

As seen in experiments 12 and 13 (table 6) if in normal animals with sugar infusion relatively large amounts of adrenalin were given in single intramuscular injections, the liver glycogen remained practically constant, i.e., the adrenalin prevented the glycogen storage from dextrose. When insulin was given previous to the administration of adrenalin and also simultaneously with it, no storage occurred (expt. 14, table 6), which would have been expected if insulin could prevent the action of adrenalin.

C. *Effect of insulin on liver glycogen in depancreatized animals.* Since, in the experiments described above, insulin caused a breakdown in liver glycogen or at least a retardation in glycogen storage, the question came up whether this was not produced by the excessive amounts of insulin. It might be assumed that the endogenous supply of insulin is always at an optimum for glycogen storage in normal animals, and that any excess would cause the opposite effect, i.e., a breakdown. We know of no analogy for this and consider the idea improbable. However, we have carried out a further series of experiments in which the pancreas was removed at the time of the experiment, and along with the sugar infusion small amounts of insulin were infused to replace the function of the pancreas without increasing the concentration of insulin above the optimum.

TABLE 6

Morphine (↑M)-chloralose (↑C). At ↑D dextrose infusion into jugular vein started. Rate: 120 cc. 10 per cent solution/hour. At ↑Ad adrenalin (3 cc. 1:1000) injected intramuscularly and repeated every 15 minutes. At ↑I insulin 4 units in 10 cc. saline injected intravenously, followed by continuous intravenous infusion of 4 units/hour.

	TIME (HOURS)						
	0	½	1	1½	2	2½	3
Expt. 12 (Dog 15.1 kgm.):							
Blood sugar	82	92		147	227	290	
Liver glycogen	↑M	↑C	1.55 ↑D ↑Ad		1.54	1.32	
Expt. 13 (Dog 15.0 kgm.):							
Blood sugar	83	85	93		225		294
Liver glycogen	↑M	↑C	7.93 ↑D, Ad		7.88		7.23
Expt. 14 (Dog 18.0 kgm.):							
Blood sugar	96	155			211		208
Liver glycogen	↑M	↑C	2.05 ↑I, D, Ad		1.41		1.79

When the pancreas was removed and neither sugar nor insulin was infused, a great fall in liver glycogen occurred (expt. 15, table 7); with sugar infusion (expt. 16, table 7) or sugar and insulin infusion (expt. 17, table 7), a fall also occurred.

We do not know the real cause of this rapid breakdown which occurred regularly in these experiments. It was thought that perhaps an excessive amount of insulin was squeezed out of the pancreas during the operative procedure, but clamping of the pancreatic veins, before the pancreas was even touched, did not prevent the breakdown. The unavoidable handling of the intestines might set up some sympathetic stimulation, which results in the rapid glycogenolysis. In order to allow the animal to overcome the

traumatic effect of the operation, the pancreas was removed in another series with sterile precautions at least 48 hours or more before the actual

TABLE 7

Morphine (↑M)-chloralose (↑C). At ↑P pancreatectomy completed, at ↑D dextrose, at ↑S saline, at ↑I insulin (crystallized) intravenous infusion started. Rate: saline 120 cc. per hour, dextrose 120 cc. 10 per cent solution per hour, insulin 4 units per hour.

	TIME (HOURS)								
	0	½	1	1½	2	3	4	5	6
Expt. 15 (Dog 16.2 kgm.):									
Blood sugar			80		180	155	144	160	175
Liver glycogen	↑M	↑C	5.72 ↑P	↑S	4.00	4.03	3.28	0.87	0.84
Expt. 16 (Dog 13.7 kgm.):									
Blood sugar			203		465	596	506	500	526
Liver glycogen	↑M	↑C	3 51 ↑P	↑D	2.71	1.91	1.70	1.01	1.67
Expt. 17 (Dog 17.3 kgm.):									
Blood sugar	92	124			195	203	217	240	268
Liver glycogen	↑M	↑C	3.78 ↑P	↑D ↑I	2 79	2 58	2 67	2 01	2 01

TABLE 8

Pancreatectomy in experiments 18, 20; 48 hours, in experiment 19, 65 hours previous to experiment. During this preliminary period in experiment 20, animal kept on sugar and insulin régime, in experiment 18, 19 neither food nor insulin was given. At ↑M morphine, at ↑C chloralose (0.08 gm./kgm.) given. At ↑D dextrose, at ↑I insulin intravenous infusion started. Rate: dextrose 120 cc. 10 per cent solution/hour, insulin (Lilly) 4 units in 10 cc. saline/hour.

	TIME (HOURS)							
	0	½	1	2	3	4	5	6
Expt. 18 (Dog 13.2 kgm.):								
Blood sugar	295	312		495	606	658	689	773
Liver glycogen	↑M	↑C	0.07 ↑D, I	0.09	0.16	0.28	0.43	1.26
	0	½	1	2½	4	5½	7	8
Expt. 19 (Dog 15.1 kgm.):								
Blood sugar	306	315		405	504	603	753	807
Liver glycogen	↑M	↑C	0.09 ↑D, I	0.16	0.27	0.96	1.64	3.31
	0	½	1	2	3	4	5	6
Expt. 20 (Dog 16.2 kgm.):								
Blood sugar	92	137		267	382	335	394	302
Liver glycogen	↑M	↑C	2.98 ↑D, I	3.37	2.10	3.03	3.72	4.46

experiments. If the pancreatectomy was performed more than 48 hours previous to the experiment, the animal was kept as a rule on a sugar and

insulin régime either up to the time of the experiment or up to 48 hours before the experiment, depending upon whether a glycogen-free liver was desired. If the operation was performed 48 hours before the actual experiment, the animal was either kept on a sugar and insulin régime or as in most cases was not given either sugar or insulin. In every instance in the actual experiment, when sugar and insulin were infused, an increase in liver glycogen occurred. In experiments 18 and 19 (table 8) neither food nor insulin was given to the animal 48 and 65 hours, respectively, previous to the experiment and as seen at the beginning, the liver is practically glycogen free. Then a gradual rise in glycogen occurs as the infusion begins. In 5 hours it reaches the value of 1.3 per cent and in 7 hours 3.3 per cent.

In experiment 20 (table 8) the animal was on a sugar and insulin régime and at the beginning of the actual experiment the liver glycogen and blood sugar values were similar to those of a normal animal. During the course of the experiment there is a rise in liver glycogen, which shows that insulin brings about a glycogen storage in diabetics, whether they are entirely free of glycogen and insulin or not.

We conclude from the above experiments that in pancreatectomized animals,—if some time is allowed for the animal to overcome the operative shock,—insulin with sugar brings about a rise in liver glycogen from the beginning of the experiment, and that this is a more or less gradual one.

Discussion. In the present paper we have presented an experimental technic for the study of glycogen formation and breakdown. Though we use an anesthetic (morphine-chloralose) and perform a laparotomy, cut and ligate the liver, we still obtain in all instances a glycogen storage, if we infuse dextrose continuously. The distribution of glycogen in the liver remains uniform. The anesthetic causes some glycogenolysis, but our basic liver sample is taken after the introduction of the narcotic and this does not prevent the glycogen storage in the later stages. On the other hand the advantages of our technic are 1, that in using large animals, many fair sized liver samples can be taken for analysis; 2, we know the glycogen content to start with and do not have to depend on assumptions which are often erroneous. Many investigators attempt to start with a liver which has been made glycogen-free through starvation. The time period of starvation varies with different observers. Some feel that a 48 hour fast is sufficient to render the liver of rabbits glycogen-free; others claim that 4 days are required. Again references are seen where 5 or 6 days are considered necessary to obtain this condition. There is no uniformity of opinion as to the number of days required to render a liver glycogen-free and a chance of mistake in this starting point renders the conclusions derived from the experiments doubtful. Besides, the starvation of an animal for a period necessary to render the liver glycogen-free

causes abnormal conditions from the start. The physiological significance of liver glycogen is not known yet, but it is known that the liver becomes glycogen-free only in rare instances and when these occur, the animal is certainly not in a physiological state. 3. We can use well fed animals, a fact which in our opinion is essential, unless one wishes to study the glycogen formation after starvation. 4. We can follow the changes hourly or even more frequently if necessary. 5. The animal is quiet throughout the experiment. 6. We do not have to introduce any new factor at the end of the experiment, as some investigators do when giving an anesthetic in order to avoid any possible liver glycogen breakdown due to excitement accompanying the operative procedure in obtaining the liver sample.

In reviewing our experiments with insulin, we see that in a diabetic dog insulin with sugar brings about a rise in liver glycogen and this occurs from the very beginning and is a more or less gradual one. On the other hand in a normal dog insulin with sugar produces a decrease in liver glycogen or at least prevents storage. If given with adrenalin it does not prevent the glycogenolytic action of the latter. We do not think that these differences can be explained on the assumption that insulin causes glycogen storage if present at an optimum amount and breakdown if in excess. We must assume, therefore, that insulin has two effects, first the breakdown of liver glycogen, which is due to a direct liver action as seen in normal animals even if there is no hypoglycemia present and even when there is a steady rise in blood sugar, and as also seen in the perfused isolated liver of normal animals. The second is the storage which may be a direct or indirect action in which latter case it may change the general metabolism in such a manner that the liver cells regain their normal storing function, or perhaps are supplied with the proper storing material.

We plan further perfusion experiments on isolated livers from diabetic dogs which should throw further light on the matter.

SUMMARY

1. A technic is described for the study of glycogen storage and breakdown in the liver.

2. In a normal dog under the experimental conditions described with no sugar infusion there occurs a steady decrease in liver glycogen. With a continuous sugar infusion, on the other hand, a rise in liver glycogen occurs. In both instances the distribution of glycogen in the liver remains uniform.

3. In a normal dog with sugar infusion and relatively large amounts of insulin given in single injections, the liver glycogen falls. With continuous slow infusion of insulin in small but effective amounts, the storage is prevented.

4. Insulin does not prevent the action of adrenalin on liver glycogen.

5. In depancreatized animals, if the pancreas is removed at the time of

the experiment, a fall in liver glycogen occurs whether sugar alone or sugar and insulin are infused or not.

6. In diabetic animals, if the pancreatectomy was performed at least 48 hours before the experiment, insulin with sugar brings about a more or less gradual rise in liver glycogen from the very beginning of the experiment.

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LIVER GLYCOGEN STORAGE IN DIABETIC ANIMALS[1]

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In a previous paper (Bodo and Neuwirth, 1933) it has been shown that in dogs in morphine-chloralose anesthesia, with the operative technic necessary in obtaining the liver samples, a rise in liver glycogen occurred in a 5 to 7 hour experiment when dextrose was infused continuously into the the circulation. When with the sugar insulin was given, either in large doses as single injections or in small but effective amount by continuous infusion, a decrease in liver glycogen or at least a retardation of storage occurred. With the insulin there was at no time a hypoglycemia, the blood sugar being well above normal and rising steadily throughout. It was shown also that in depancreatized dogs not fed for 48 to 65 hours before the experiment, when sugar and insulin were infused, a storage of liver glycogen occurred. The glycogen formation was a more or less gradual one, that is, there was no preliminary latent period before storage, as might have been expected. The glycogen content of the liver increased from practically 0 to 1.3 per cent in 5 hours and to 3.3 per cent in 7 hours. From these experiments it was thought that insulin was an indispensable factor in liver glycogen formation. The paper now to be presented throws doubt on this assumption.

Methods. All the experiments were carried out on dogs from which the total pancreas was removed at least 48 hours previous to the actual experiment. When the pancreatectomy was performed more than 48 hours previous to the experiment, the animals were kept on a sugar and insulin régime. The insulin was discontinued 48 hours before the experiment, but the sugar régime varied in the different series.

The actual experiment was performed either in morphine and chloralose anesthesia or without anesthesia. For the morphine-chloralose anesthesia,

[1] This paper was read at the annual meeting of the Federation of American Societies for Experimental Biology in Philadelphia, April 1932, and the abstract appeared in THIS JOURNAL, ci, Proc. 10, 1932. After we had sent in our manuscript there was published in the November number of THIS JOURNAL (1932 C 11, 409) a paper by S. G. Major and F. C. Mann, which confirms our results though the increase in liver glycogen was smaller than in our experiments.

morphine sulphate 3 mgm. per kgm. body weight subcutaneously and chloralose 0.07–0.10 gram per kgm. body weight intravenously were used. If necessary, after 4 to 5 hours' infusion, a second small dose of chloralose (0.3 gm.) was injected intravenously. Samples of liver (about 5 grams each) were cut out at 90 to 100 minute intervals in the same way as described in the previous paper. The glycogen determination differed from the one described before, in so far that 99 per cent alcohol was used for precipitation in such amounts as to obtain a final alcoholic concentration of more than 70 per cent. The methods and technic employed otherwise were exactly the same.

A. *Effect of sugar infusion on liver glycogen in diabetic animals under morphine-chloralose anesthesia.* In the first series of experiments the completely pancreatectomized animals were given neither food nor insulin during the 48 hours previous to the actual experiment. At the end of this

TABLE 1

Pancreatectomy 48 hours previous to experiment. Neither food nor insulin given during these 48 hours. At ↑ M morphine, at ↑ C chloralose (0.07 gm./kgm.) given. At ↑ D dextrose intravenous infusion started. Rate: 120 cc. 10 per cent dextrose solution/hour. No insulin.

	TIMES (HOURS)						
	0	½	1	2½	4½	6½	8
Expt. 1 (Dog 15.0 kgm.):							
Blood sugar, mgm. per cent.	324	324		366	560	828	1,156
Liver glycogen, per cent...	↑ M	↑ C	0.17 ↑ D	0.26	0.22	0.47	1.06
Expt. 2 (Dog 15.5 kgm.):							
Blood sugar.....		258		588	738	942	1,146
Liver glycogen.....	↑ M	↑ C	0.18 ↑ D	0.28	0.42	0.58	0.97

time under morphine and chloralose anesthesia, dextrose was infused intravenously for a period of 7 hours (expts. 1 and 2, table 1). Contrary to expectation there occurred a rise in liver glycogen from practically 0 to approximately 1 per cent. This corresponds to the rise which had been obtained in 5 hours when insulin was infused with the sugar (Bodo and Neuwirth, 1931). The blood sugar with no insulin increased to 1.1 per cent in 7 hours, whereas with insulin it reached only 0.7 to 0.8 per cent (Bodo and Neuwirth, 1931). The water content of the liver remained unchanged throughout the experiment.

B. *Effect of subcutaneous sugar injections on liver glycogen in diabetic animals without anesthesia.* The above results could not be easily reconciled to the well-known picture of the diabetic animal in which the liver is glycogen free in spite of the high blood sugar values. In order to show that the anesthetic was not a responsible factor (it has in fact, as has been shown,

an opposite effect) we repeated the experiments just described with the difference that after the 48 hours, during which they were not given either food or insulin, the animals received dextrose subcutaneously at hourly intervals for 5 hours without anesthesia. At the end of this time liver samples were taken either in chloralose or in local anesthesia. The results in terms of liver glycogen were practically the same, that is, about 1 per cent (table 2).

In another series of experiments, during the 48 hours following the removal of the pancreas, subcutaneous injections of dextrose were made

TABLE 2

Pancreatectomy 48 hours previous to experiment. Neither food nor insulin given during these 48 hours. Then 300 cc. 10 per cent dextrose solution subcutaneously at hourly intervals for 5 hours without anesthesia. At the end of this time liver samples taken for analysis either under chloralose or local anesthesia.

	GLYCOGEN
	per cent
Dog 1 Lobe 1	0.58
Dog 1 Lobe 2	0.52
Dog 2 Lobe 1	0.97
Dog 2 Lobe 2	0.92

TABLE 3

Pancreatectomy. Following this, subcutaneous injections of 300 cc. 6 per cent dextrose solution twice a day for 48 hours. At end of 48 hours liver samples taken for analysis under chloralose or local anesthesia.

	LIVER GLYCOGEN
	per cent
Dog I	0.90
Dog II	0.73
Dog III	0.58
Dog IV	0.97

twice daily. At the end of the 48 hour period liver samples were taken and again found to contain about 1 per cent glycogen (table 3). It should be kept in mind that if no sugar had been given, the liver would be practically glycogen free.

C. *Effect of sugar infusion on liver glycogen in diabetic animals after sugar régime.* In the fourth series of experiments, as in the third, during the 48 hours after removal of the pancreas subcutaneous injections of dextrose were made twice daily, but at the end of the 48 hours an additional infusion of sugar, under anesthesia, was made for a period of 7 hours. The liver

glycogen was about 0.7 per cent at the beginning of this period and showed only a slight rise during the 7 hours (expt. 3, table 4). At the five hour period the two figures indicate two different liver lobes. It would seem that about 1 per cent glycogen represents the maximum if no insulin is added.

D. *Effect of sugar and insulin infusion on liver glycogen in diabetic animals after sugar régime.* When the procedure was the same as in experiment 3, except that insulin was added to the sugar infusion (expt. 4, table 4), it is seen that the liver glycogen rose to 3.6 per cent in the 7 hour infusion.

E. *Effect of sudden rise of blood sugar on liver glycogen in diabetic animals.* In our experiments the diabetic animals had a high blood sugar value and no glycogen in the liver, and whenever the blood sugar was further in-

TABLE 4

Pancreatectomy 48 hours previous to experiment. Subcutaneous injections of dextrose during these 48 hours (no insulin). At ↑M morphine, at ↑C chloralose (0.07 gm./kgm.) given. At ↑D dextrose, at ↑I insulin intravenous infusion started. Rate: dextrose 120 cc. 10 per cent solution/hour, Insulin (Lilly) 4 units/hour. In experiment 3 no insulin infusion.

	TIME (HOURS)						
	0	½	1	2½	4½	6½	8
Expt. 3 (Dog 13.2 kgm.):							
Blood sugar.....	500	500		896	1,030	1,220	1,300
Liver glycogen...	↑M	↑C	0.73 ↑D	0.71	1.04	1.21 0.98	0.92
Expt. 4 (Dog 10.4 kgm.):							
Blood sugar.....	295	309		495	693	846	891
Liver glycogen...	↑M	↑C	0.45 ↑D, I	0.70	1.17	2.27	3.59

creased by infusion or injection, glycogen was deposited. This was specially marked when the blood sugar reached 0.8 to 1.1 per cent. Considering these facts one could assume that the glycogen storage depends entirely on a certain threshold value of sugar. It is quite conceivable that in normal animals without anesthesia this threshold value is perhaps somewhere around 0.15 per cent, in anesthesia somewhat higher, in diabetics with insulin around 0.6 to 0.7 per cent, and in diabetics without insulin around 0.8 to 1.1 per cent. In connection with this, we would like to mention that in the perfusion experiments on the isolated liver (Bodo and Marks, 1928) glycogen storage was obtained only if the blood sugar was between 0.5 and 1.0 per cent.

In order to see whether there is a certain blood sugar threshold required for glycogen storage in the diabetic animal without insulin, we decided to

raise the blood sugar at the beginning of the experiment suddenly to a value which it would reach only after 6 to 7 hours with slow sugar infusion. The rapid infusion of large amounts of sugar (500 cc. 10 per cent dextrose solution in ½ hour) was not sufficient because of rapid kidney excretion. Therefore, to obtain very high sugar values, the kidney vessels were ligated before the rapid, large sugar infusion. Experiment 5 (table 5) was performed in such manner, the kidney vessels were ligated and sugar infused, but even so the blood sugar value was only 0.7 per cent. As regards the liver glycogen figures, the experiment does not justify our expectation, because there was no sudden rise in liver glycogen, the storage occurring just as slowly as usual. It may be that a time factor plays an important part in the glycogen formation, that is, a certain time is necessary for the storage regardless of the rapidity with which the blood sugar threshold is exceeded.

TABLE 5

Pancreatectomy 8 days previous to experiment. Sugar and insulin régime up to 48 hours before experiment. At ↑ M morphine, at ↑ C chloralose (0.07 gm./kgm.) given. At ↑ K kidney vessels ligated. At ↑ D1 dextrose intravenous infusion started. Rate: 400 cc. 10 per cent dextrose solution/hour. At ↑ D2 rate of dextrose infusion changed to 120 cc. 10 per cent solution/hour.

	TIME (HOURS)							
	0	½	1	1½	2	3	5	6
Expt. 5 (Dog 14.5 kgm.):								
Blood sugar	360	340			700	1,000	1,570	1,700
Liver glycogen	↑ M	↑ C	0.17	↑ K, ↑ D1	0.21	0.27 ↑ D2	0.43	0.63

DISCUSSION. In reviewing our experiments we see that if the pancreas is completely removed from dogs, and during the 48 hours previous to the experiment the animals are not given either food or insulin, the liver is practically free of glycogen. If at this time sugar is infused or injected, with or without anesthesia, a rise in liver glycogen occurs, which means that in diabetic animals without the administration of insulin a glycogen storage can occur.

Without the addition of insulin we could not obtain more than about 1 per cent glycogen in the liver in the 7 hour experiments, whereas with insulin during the same time, the glycogen amounted to approximately 3.6 per cent.

Even by infusing suddenly large amounts of sugar, we could not produce a sudden rise in liver glycogen.

We cannot offer a satisfactory explanation for these results. If insulin is indispensable for glycogen formation, then it must be assumed that it is

still present 48 hours after pancreatectomy. This assumption is against general opinion. Even if it is present, it is not present in sufficient quantity, because it is unable to bring about glycogen storage from the large amount of blood sugar available, but requires additional sugar for this purpose. That insulin alone without the addition of sugar is capable of producing glycogen storage in diabetic animals, has been shown by Cori (1925) and by Hédon (1925).

There is another possibility, that it is not the amount but the kind of sugar that determines glycogen storage, which implies that the diabetic animal's own sugar is not directly available for glycogen formation. There is no experimental evidence bearing on this.

Macleod (1927) in a recent paper states that perhaps the most important function of insulin is that it brings about glycogen storage, and if there is no insulin, there cannot be any glycogen formation unless there is sugar present which has "passed through the glycogen stage" in the liver. If one would accept Macleod's conception we must assume from our experiments first, that the liver was not glycogen free previous to the actual experiment, but became free on account of the anesthesia, the sugar resulting from the glycogen breakdown being then available for synthesis, or second, that there was in the circulation sugar which had once "passed the glycogen stage" but was not stored as glycogen until more dextrose was added. Against the first assumption we would point out that there was no glycogen in the liver when the samples were taken under local anesthesia or in chloralose anesthesia without morphine (morphine causes the breakdown, not chloralose). The second assumption, though it is possible, is not probable.

We are inclined to believe that glycogen storage can occur in the absence of insulin. The insulin action may be conceived then, not as an essential factor, but as more of the nature of a catalytic one in accelerating or increasing glycogen storage just as it is believed to act on the oxidations in muscle. From this standpoint, the absence of insulin may explain the larger amount of sugar required even for the moderate degree of glycogen storage which we have found.

SUMMARY

1. In completely depancreatized animals, if neither food nor sugar is given for 48 hours, the liver is glycogen free.

2. In diabetic animals when the liver is glycogen free, with the administration of sugar alone, with or without anesthesia, glycogen storage occurs in the liver, up to a maximum of about 1 per cent.

3. In diabetic animals with the administration of sugar and insulin a greater degree of glycogen formation occurs.

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FRESH AND DRIED YEAST AS SOURCES OF VITAMIN B[1]

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From Food and Drug Administration, and the Bureau of Chemistry and Soils, U. S. Department of Agriculture

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During recent years considerable attention has been drawn to yeast through extensive advertising. In addition to yeast in various forms there have appeared on the market also numerous products alleged to contain extracts or the vitamins of yeast. Investigations of the vitamin content of this class of products have been in progress in connection with the enforcement of the Federal food and drugs act. In the course of the studies on fresh yeast cakes it was found that if the material is dried before feeding, the amount of vitamin B available to the rat is materially increased. This fact has not been previously observed or recorded so far as we are aware and is of interest not only because of its application to the alleged therapeutic value of fresh yeast, but also because of its possible application in considering the nutritive value of plant material containing living cells.

Vitamin B determinations were being made on small fresh compressed yeast cakes which contain starchy material and are wrapped in tin-foil. They will be referred to hereinafter as fresh yeast cakes. It was decided that these studies could be facilitated if these cakes were dried. Notwithstanding the fact that there is adequate evidence that vitamin B is a rather stable substance, it was considered necessary to determine whether this vitamin in fresh yeast cakes is injured by drying the cakes at room temperature. Fresh yeast cakes were purchased daily, and a portion of this material was fed to rats on a vitamin B deficient ration. The remainder was dried at room temperature before an electric fan and ground. The dry material, hereafter referred to as dried yeast cake no. 1, was fed in quantities equivalent to the fresh yeast in duplicate vitamin B experiments.

In the absence of a recognized standard for vitamin B, such as the international standard recently recommended by the Health Committee of the League of Nations (Editorial 1931) it was decided to use for comparison a

[1] Prior to September 1, 1931, when the Food and Drug Administration established its Nutrition Laboratory, investigations of this character were conducted for the Administration by the Protein and Nutrition Division of the Bureau of Chemistry and Soils. These investigations were made in connection with the enforcement of the Federal food and drugs act.

representative sample of hard wheat. The grain used was a composite of samples of hard red winter wheat[2] from 25 elevators in the State of Nebraska.

Male rats about four weeks old, weighing approximately 60 grams, were used. They were kept in individual cages with screen bottoms and allowed to eat the following rations *ad libitum*: Commercial casein extracted with hot alcohol, 18 parts; Osborne and Mendel's salt mixture, 4 parts; agar, 2 parts; dextrinized cornstarch, 67 parts; cod liver oil, 2 parts; and yeast autoclaved 4 hours at 15 pounds' pressure and shown to be devoid of demonstrable traces of vitamin B, 5 parts. The cod liver oil was added to only such quantities of the basal ration as would be consumed within a period of one week. The products tested for vitamin B were placed daily before the animals in small glass caster cups.

On the basis of available information it was assumed that the average weight of one fresh yeast cake was 14.1 grams. This value was found to be high for most of the cakes as purchased, due to rapid loss of moisture. However, no correction was made for the variations from this value, inasmuch as the variations have no significant influence on the interpretations of the results.

Fresh yeast cakes dried at room temperature lost about 68 per cent of moisture and the fractional portions of the dried yeast cakes were calculated on this basis.

The left half of chart I shows the growth response of animals fed two levels of fresh yeast cake, the same quantities of that material dried, wheat equal in weight to $\frac{1}{16}$ fresh yeast cake, and animals receiving no vitamin B. It is apparent from the chart that $\frac{1}{8}$ of a cake of fresh yeast, $\frac{1}{16}$ of a cake of the same material dried, and wheat equal in weight to $\frac{1}{16}$ of a fresh yeast cake, are approximately equal as sources of vitamin B to the rat. Therefore, twice as much vitamin B could be obtained from a yeast cake that had been dried as from a fresh cake, and one cake of fresh yeast provided no more vitamin B than one-half of its weight of wheat.

The results of the vitamin B determinations were unexpected and led to a further study to confirm these findings. In order to dry the fresh yeast cake rapidly under carefully controlled conditions, some of the material was dried in a current of filtered air for three hours at a temperature of 50°C. Fresh yeast cake dried in this manner is referred to as dried yeast cake no. 2. A cake of fresh yeast was purchased daily, and $\frac{1}{16}$ of it was fed to each of the rats in lot B-35, chart I. The remainder of the cake was dried the same day. A composite sample was made of the special dried yeast cake prepared in one week and fed the following week to lot B-36 in quantities equal to one-half of the fresh yeast fed lot B-35. Lot B-37 was fed 0.440

[2] Kindly furnished by D. A. Coleman, Ph.D., Bureau of Agricultural Economics, U. S. Department of Agriculture.

gram of ground hard wheat. It is possible to bring out more accurately differences of vitamin B potency by restricting the quantity of vitamin B fed to a level that permits a slow rate of growth. In view of his fact, the daily feedings of yeast and wheat were reduced to one-half of those pre-

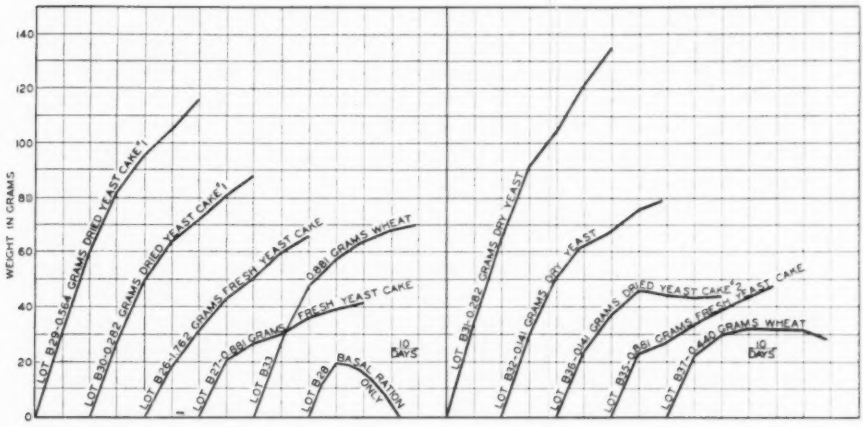

Chart I. The whole and fractional portions of the fresh yeast cake in terms of their equivalent weights when fresh and dried are shown in the following table:

	When fresh *grams*	When dried *grams*
Weight of 1 yeast cake	14.100	4.512
Weight of $\frac{1}{8}$ yeast cake	1.762	0.564
Weight of $\frac{1}{16}$ yeast cake	0.881	0.282
Weight of $\frac{1}{32}$ yeast cake	0.440	0.141

Each curve on this chart represents the average growth of four male rats, each rat receiving daily the quantity of material indicated as a sole source of vitamin B. It is apparent from the response of lots B-29, B-30, B-26, and B-27, that more than twice as much vitamin B is available to the rat from the yeast cake which has been dried as from the fresh material. Since lot B-33 grew fully as well as lot B-26, wheat must be considered twice as good a source of vitamin B as fresh yeast cake. A comparison of lots B-29 and B-30 with lots B-31 and B-32 shows that there is approximately twice as much vitamin B available from a commercial dry yeast as from the dried yeast cake material used. Further evidence of this relationship is provided in relative growth responses of lots B-32 and B-36. One thirty-second of a cake of dried yeast cake no. 2 was found to be approximately equal to $\frac{1}{16}$ of the same cake of fresh yeast as a source of vitamin B.

viously used. As shown in the right half of chart I, the results obtained substantiate the previous findings.

It was also of interest to compare dried yeast cake with a commercial dry yeast whose vitamin B potency had previously been established. In

order to make the results directly comparable, the yeasts were both fed at similar levels. The dried yeast cake showed a vitamin B potency about one-half as great as the commercial dry yeast. The proportion of starchy material which the dried yeast cake contained was sufficient to account for the difference in vitamin B potency of the two preparations and indicate that the yeasts were approximately equal in vitamin B potency.

Discussion. In seeking a possible explanation of the results of vitamin B determinations on the various yeast preparations, it was thought that the number of viable yeast cells might bear some relationship to vitamin B potency. It was found that the preparations most potent in vitamin B contained the lowest proportion of viable yeast cells, and conversely the preparations that contained the largest proportion of viable yeast cells were the least potent in vitamin B. However, the physical changes which take place in a yeast cell due to drying may be of such a nature as to make vitamin B more readily available even though the cell is not actually killed.

After these studies were completed, the authors became familiar with the investigations of Montgomery, Boor, Arnold, and Bergeim (1931) showing that from 55 to 95 per cent of the number of live yeast cells ingested may pass through the stomach without being destroyed. That observation suggests an explanation of the inability of the rat to obtain as much vitamin B from fresh yeast as from the same material when dried. Presumably the live yeast cell offers sufficient resistance to digestion to prevent the rat from obtaining the full nutritive value of the yeast. Additional evidence has been obtained which shows that this applies to substances other than vitamin B. The factors that are operative in preventing rapid digestion and assimilation of live yeast cells and possibly the living cells of other plant tissue should be established by further investigations. The digestive mechanisms of a carnivorous animal may limit still further his ability to obtain all of the nutrients of raw vegetable material. Compilations of the vitamin content of foods (Smith, 1929; Sherman, 1931) indicate that the omnivorous rat, which is used most frequently in vitamin B determinations, is able to obtain as much of that vitamin from living vegetable cells as from prepared foods, but if the determinations on which these compilations are based are sufficiently accurate, yeast must be considered an exception. The growth responses show that the yeast cells of the fresh yeast cake are not as good a source of vitamin B for growth as the yeast cells of the dried yeast cake, and therefore other physiological effects which have been specifically attributed to vitamin B, such as stimulation of the appetite (Karr, 1920; Cowgill, Deuel and Smith, 1925), and relief from gastric atony (Cowgill, Deuel, Plummer and Messer, 1926) cannot be expected to follow ingestion of fresh yeast cake to a greater degree than did the observed growth responses.

SUMMARY

Data are provided to show that the amount of vitamin B obtained by the rat from fresh compressed yeast cakes is approximately half that obtained from a similar cake that has been dried. These results may have a bearing on the vitamin determinations of plant material containing living cells.

Data are provided to show the relative vitamin B potency of commercial fresh compressed yeast cakes, the same material dried, a commercial dry yeast, and a composite sample of Nebraska hard wheat.

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THE EFFECT ON LACTATING MICE OF INJECTING AN EXTRACT OF THE URINE OF PREGNANCY

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Recent studies on the hormonal control of lactation have chiefly been concerned with the induction of lactation or mammary growth in non-lactating animals (*cf.* Stricker and Grueter, 1928, 1929; Parkes, 1929; Corner, 1930; Frank and Turner, 1930; Nelson and Pfiffner, 1930, 1931). It seemed not unlikely that the mammary hyperplasia and milk secretion accompanying gestation and parturition might be influenced by factors different from those governing post-partum lactation. Extraordinary mammary hyperplasia and even copious milk secretion have been induced by the administration of anterior pituitary substance (Corner, 1930; Evans and Simpson, 1929, 1931) particularly extracts containing the luteinizing principle of the anterior pituitary. Asdell (1932) has reported that luteinizing hypophyseal extracts are to some extent effective in prolonging lactation in goats, and Grueter (1930) has also observed increased lactation in cows and goats with the administration of similar extracts. It seemed, therefore, that an effectively luteinizing extract of pregnancy urine might be a potent stimulant to increased milk production.

We obtained, accordingly, through the kindness of Dr. E. A. Sharpe of Parke, Davis & Co., a preparation of pregnancy urine (Antuitrin S) which upon our own assay as well as that of Parke, Davis & Co., demonstrated a definite luteinizing effect. On the assumption that any influence on lactation would be reflected in the growth of the suckling young, daily weights were taken of the young of injected mothers. The Bagg-Little strain of albino mice was used since the post-natal growth curve for the strain has been carefully determined in this laboratory. For each set of injections five females were employed, the litter of each female being reduced to four at birth to permit comparison with our standard curve. Each mother was injected daily from the day of the birth of the young until twenty to twenty-one days after the birth date. The dosages varied from 0.1 to 10 rat units in $\frac{1}{10}$ cc. of normal saline; one group of females used as controls received daily injections of normal saline solution.

The data are summarized in figures 1 to 8 (we have omitted the data for

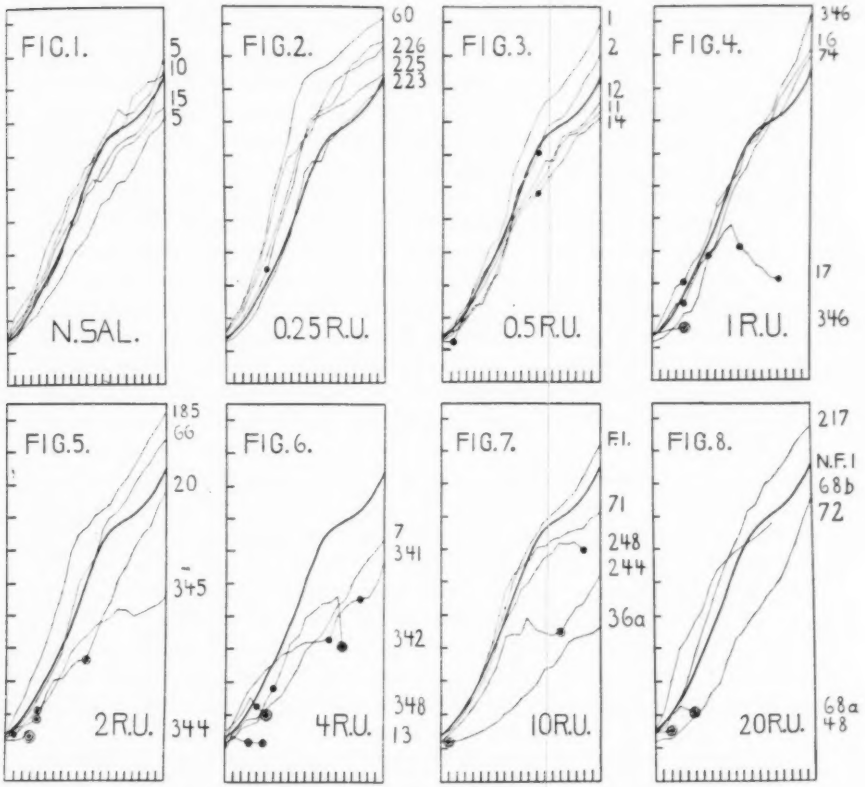

Fig. 1. The heavy black line, (labelled *NF* in fig. 8) in this and all succeeding figures, represents the standard growth curve (males and females combined) for the strain of mice employed. In this and all succeeding figures the numbers at the right are our record numbers of the mothers employed. Abscissa = days, ordinate = weight in grams. Normal saline injected daily for 20 days. No deaths.

Fig. 2. Twenty-five hundredths unit of antuitrin injected daily. The preponderance of heavier young is due to the use of mothers somewhat older than those used for producing young for our standard curve. One death.

Fig. 3. Five-tenths unit of antuitrin injected daily. Note slight depression of growth rate. Three deaths.

Fig. 4. One unit of antuitrin injected daily. Note alteration of growth curve. Eleven deaths.

Fig. 5. Two units of antuitrin injected daily. Growth curves altered. Eleven deaths.

Fig. 6. Four units of antuitrin injected daily. Fifteen deaths.

Fig. 7. Ten units of antuitrin injected daily. Ten (?) deaths. Injections on no. 248 discontinued because of weakness of young.

Fig. 8. Twenty units of antuitrin injected daily. Twelve (?) deaths. Injections on no. 72 discontinued because of weakness of young.

injections of 0.1 unit as they differ in no way from those for normal saline injections). The heavy line in each figure represents the standard curve for this strain. Each symbol represents the death of one individual.

Two general effects are observed: 1, with increasing dosages the growth curve of the young is depressed and somewhat altered; 2, with increasing dosages the number of young dying increases.

Both effects observed are obviously due to the failure of the milk supply of the mothers. This is deducible from the fact that most of the deaths occur during the first two weeks after birth when the young are entirely dependent on the mother's milk, the few deaths occurring after that period being due in part to a general weakness resulting from the previous poor nutrition. It will be noticed further that the average growth rate for the litter increases for a short time after the death of one or two of the young. This is due not merely to the increase in average weight due to the death of presumably the lightest mouse in the litter, but also to the fact that more milk is available to the remaining young. In some cases, indeed, the recovery from the two weeks of poor milk supply is so marked that the break in the standard curve occurring at 14 days after birth is entirely obliterated. We have, in fact, in other experiments designed to limit nutrition in the first few days after birth, observed a similar elimination of this break in the growth curve. The theoretical implications of this alteration of the growth curve will be presented by one of us elsewhere. Immediately it is obvious that the administration of pregnancy urine extracts to nursing mothers has a definitely harmful effect upon the young. Our observations indicate no harmful effect on the mothers which remained in good condition throughout the experiment.

Our observations indicate that the number of young killed is approximately proportional to the logarithm of the number of hormone units injected. Exact calculations of the lethal effect are somewhat difficult to make because older mothers appear to be somewhat more resistant than younger mothers. It is interesting to note that although ovarian weight increases very slightly in response to even very large increases in dosage of pregnancy urine extracts (*cf.* Evans, Meyer, and Simpson, 1931), the lethal effect described here is proportionate to the dosage.

The females of the strain used are generally very good mothers, the loss of young in our general stock being rather rare. The lethal effect can therefore be attributed directly to the hormone preparation employed as, indeed, the loss of no young in either the saline injected or the 0.1 unit series attests. It is possible that some factor other than the ovary-stimulating hormone in the preparation was responsible for the general effect. This seems unlikely, however, as in a few instances we employed preparations which as a result of long standing had lost potency, and in these cases the effect had with the fresh preparations was not observed. In any event

our observations give no indication as to whether the hormone acted directly on the mammary glands or through the mediation of the ovary. In the latter case the possibility exists that sufficient oestrogenic potency was contained in the preparations to stimulate oestrin production by the ovary, and that the effect is due to the action of this oestrin on the mammary glands. Parkes and Bellerby (1927), however, found no impairment of lactating efficiency on the injection of fairly large doses of oestrin into lactating mice beyond a slight break in the growth curve of the young.

SUMMARY

Pregnancy urine extracts (antuitrin) potent as agents in ovarian luteinization, exert definite deleterious effects on lactating efficiency when continuously injected into nursing mice. These effects are exhibited in a reduced growth rate and the death of young. The number of young dying is roughly proportional to the logarithm of the hormone dosage.

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ON THE PROTEIN CONTENT AND NORMAL FLOW OF LYMPH FROM THE FOOT OF THE DOG[1]

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Direct efforts to measure the amount of lymph produced from a limited tissue area during definite periods of time are not numerous and are as unsatisfactory as they are scarce. Emminghaus (1873) cannulated a hind-foot lymphatic exactly as we have done. His animals had large doses of opium and were absolutely quiescent during lymph collection. Lymph flow was secured by massage. A table of the amounts of lymph obtained in 10 minute periods is of some interest when compared with our figures. No data on protein concentration are given. Paschutin (1872) a year previously, made an elaborate series of experiments involving the foreleg of the dog, but complications due to curare and opium do not permit comparison of his data with our own. Hamburger (1894) cannulated a lymphatic in the neck of the horse. His figures are of interest for the constancy of flow which occurred when the animal remained under steady conditions, but the drainage area served by the cannulated lymphatic is so indefinite as to fail to give a measurement of lymph production from a limited region during a definite time. Winternitz (1895) cannulated a groin lymphatic in large dogs heavily narcotized with morphine. The lymphatic cannulated is one of a rather variable number of trunks and one cannot use the data on lymph flow in calculating lymph production in the leg.

During the past year we have found it relatively easy to collect lymph from draining trunks leading from the fore and hind feet of large dogs. The animals were unanesthetized during lymph collection and moved about the laboratory in a wholly normal manner. As a result of many injections of the foot and leg lymphatics it is possible to employ the figures obtained in making a rough approximation of the total amount of lymph produced in the foot during the period of observation. It also happens that enough lymph was secured to permit frequent refractometric estimations of the protein content, and it has been possible to follow the protein composition of lymph during periods of continuous lymph production when lymph and

[1] This investigation was aided by a grant from the DeLamar Mobile Research Fund of Harvard University.

blood capillary filtrate must be of very similar composition. One can thus gain an idea of the concentrations in which the blood proteins can pass through the normal capillary wall.

To the experiments in which the dogs have walked or run about the laboratory we have added two in which venous stasis has been produced, in order to find out whether lymph composition reflected the changes in blood capillary filtrate reported by Landis, Jonas, Angevine and Erb (1932). That in a general way this is the case will be evident from examination of protocols 3 and 4.

EXPERIMENTAL. Both fore-leg and hind-leg lymphatics have been used in our experiments. Figure 1 shows a cannula in place just above the wrist-joint in the fore-leg. Hind-leg cannulas are placed similarly just above the ankle-joint. The vessels cannulated are found through a midline incision not more than an inch in length, and are easily seen when the operator has become familiar with their appearance. Until one has had

TABLE 1

Lymph flow from the hind paw of 5 dogs narcotised with opium

Lymph flow in cubic centimeters per 10 minutes due to massage. From Emminghaus, 1873

DOG 3	DOG 8	DOG 9	DOG 11	DOG 15
1.4	0.9	0.5	1.2	0.8
0.4	0.7	0.4	0.8	0.4
0.1	0.5	0.3	0.6	0.5
0.3	0.5	0.2	0.5	0.3
0.3	0.3	0.2	0.5	0.1
0.1	0.3	0.1	0.4	0.15

experience the best way to display them is to inject 2 cc. of a 2 per cent solution of trypan blue in physiological saline under the skin of the foot. Light massage and motion at once cause the blue to enter lymphatics and flow up the leg. One may thus display all the large lymphatic trunks in the leg, the entire procedure occupying less than a minute. Trypan blue enters more readily than does a mass containing definite particles such as India ink or graphite. It diffuses out of the large lymphatics in the course of some hours, but is ideal for quick display of the vessels in any region. Ando (1930), on the suggestion of Kihara, has injected the lymphatics and nodes about the uterus with "Japanese ink" prior to panhysterectomy. We have used trypan blue to display lymphatics in the ankle of one of the authors (M. E. F.). It is wholly unirritating and fills the vessels so much more readily than ink as to have possible usefulness in surgical operations where extensive dissection of lymphatics is necessary. The method of displaying lymphatics used by ourselves and by Kihara's students is in no

sense new. Paschutin (1872) employed precisely the same procedure for filling the lymphatics in the fore-leg of the dog with Berlin blue, in order to become familiar with their distribution. The method is mentioned by other pupils of Ludwig, and was apparently in daily use in his laboratory. McMaster and Hudack (1931, 1932) and Hudack and McMaster (1931, 1932) have recently made beautiful use of the indirect method for filling capillary lymphatics with dyes in living mice, thereby making possible direct observations upon these heretofore elusive elements in the absorbing system.

The lymphatics cannulated in the following experiments, even in large dogs (animals used in our experiments weighed between 20 and 30 kgm.), are a little greater in diameter than an ordinary pin. The cannulas em-

Fig. 1. Fore-leg of dog with a cannula in a lymphatic

ployed are of quartz. They are tied in with fine silk and held in place by stay sutures through the skin. In the first experiments ether was used during the cannulation, but later it was found possible to operate with novocaine. In all cases the skin near the incision was kept infiltrated with novocaine and adrenin, so that the whole experience was entirely painless.

In every experiment we have cannulated a single vessel. Many injections of the fore- and hind-leg lymphatics have shown that at the wrist and ankle there is a concentration of vessels, and in both cases there are three, or at the very most four, draining trunks. There is no detectable difference in size and when an animal walks the flow through all should be equal. In estimating lymph flow we have multiplied the flow from a single vessel by 3, and feel the result must give a very fair approximation of the total lymph flow from the part. Protein concentrations have been measured refractometrically. Protocols of typical experiments are as follows:

March 9, 1931. Dog 1. Standing and walking. Ether anesthesia. Cannulation fore-paw lymphatic.

12:15 Ether ceased
12:23 Lymph protein 1.18 per cent
12:25 Lymph protein 1.34 per cent
12:59 Lymph protein 1.34 per cent. Dog recovering from ether. Attempts to stand. Unsuccessful
1:05 Lymph protein 1.40 per cent.
1:10 Lymph protein 1.41 per cent
1:27 Lymph protein 1.34 per cent
1:47 Lymph protein 1.34 per cent. Until this time the animal was lying down with practically no motion. Lymph specimens large enough to estimate the protein refractometrically were secured by light massage of the foot
1:47 to 1:58 Dog walked across the laboratory, into the hall and back—about 15 meters
1:56 Lymph protein 0.96 per cent
1:58 Lymph protein 0.87 per cent
2:04 Lymph protein 0.84 per cent
2:06 to 2:10 Lymph protein 0.76 per cent. Dog walked 150 meters. Flow 0.84 cc. in 4 minutes; $0.84 \times 3 \times 2.5 = 6.3$ cc. from foot in 10 minutes
2:10 to 2:20 Lymph protein 0.5 per cent. Dog standing. Entirely quiet. Flow 0.12 cc. in 10 minutes; $0.12 \times 3 = 0.36$ cc. from foot in 10 minutes
2:31 to $2:35^{30}$ Lymph protein 0.5 per cent. Dog walked 30 yards slowly. Flow 1.01 cc. in $4\frac{1}{2}$ minutes; $1.01 \times 3 \times 2.2 = 6.7$ cc. from foot in 10 minutes
2:35 to 2:55 Dog lying down and sleeping
2:55 Lymph protein 0.63 per cent
2:56 to 3:00 Lymph protein 0.56 per cent. Dog walked 30 yards slowly. Flow 0.79 cc. in 4 minutes; $0.79 \times 3 \times 2.5 = 5.9$ cc. from foot in 10 minutes
3:00 to 3:20 Lymph protein 0.59 per cent. Dog resting, lying down
3:22 to 3:37 Lymph protein 0.56 per cent. Dog trotting slowly around the laboratory building. Flow 1.29 cc. in 15 minutes; $0.86 \times 3 = 2.6$ cc. from foot in 10 minutes

March 15, 1932. Dog 2. Standing, walking and running. Effect of ether

9:40 to 10:20 Dog lying quietly on table. Left fore-paw lymphatic cannulated under novocaine
10:20 Lymph protein 1.98 per cent
10:50 Lymph protein 2.04 per cent. There was no spontaneous flow of lymph. Specimens were obtained by light massage of the foot
$11:01^{15}$ to 11:12 Effect of walking (see table 2)
11:12 to 11:30 Lymph protein 1.61 per cent. Lying quietly
11:31 to 11:38 Effect of running (see table 3)
11:55 Dog fed 0.5 kgm. lean meat
11:55 to 12:45 Dog resting quietly
12:45 Lymph protein 2.12 per cent
12:45 to 1:45 Dog resting. Occasional brief periods of motion
1:00 Lymph protein 1.90 per cent
1:45 Lymph protein 1.48 per cent

1:51 to 1:57 Effect of walking (see table 4)
1:57 to 2:02 Effect of running (see table 5)
2:10 to 2:25 Ether anesthesia to observe the effect of this anesthesia on lymph flow and composition

TABLE 2

Lymph flow and protein concentration during walking

TIME	DISTANCE TRAVELLED	LYMPH FLOW	PROTEIN CONCENTRATION
	meters	*cc.*	*per cent*
11.01[45]			
11.02	25	0.08	1.98
11.03	100	0.12	
11.04	100	0.06	
11.05	100	0.06	
11.06	100	0.05	1.54
11.07	100	0.04	
11.09	100	0.04	1.52
11.10	100	0.04	
11.11	100	0.04	
11.12	100	0.04	1.52
		0.57	

Flow 0.57 cc. in 10 minutes and 15 seconds
$0.55 \times 3 = 1.7$ cc. from foot in 10 minutes

TABLE 3

Lymph flow and protein concentration during running

TIME	DISTANCE TRAVELLED	LYMPH FLOW	PROTEIN CONCENTRATION
	meters	*cc.*	*per cent*
11.31			
11.32	200	0.01	
11.33	200	0.07	1.58
11.34	200	0.05	
11.35	200	0.05	1.42
11.36	200	0.05	
11.37	200	0.05	
11.38	200	0.05	
		0.33	

Flow 0.33 cc. in 7 minutes
$0.48 \times 3 = 1.4$ cc. from foot in 10 minutes

2:25 to 2:30 Lymph protein 1.38 per cent; 0.08 cc. of lymph produced; $0.08 \times 3 =$ 0.24 cc. total lymph
2:30 to 2:45 Lymph protein 1.34 per cent; 0.15 cc. of lymph produced; $0.15 \times 3 =$ 0.45 cc. total lymph. Dog perfectly quiet
2:45 to 3:00 Lymph protein 1.41 per cent; 0.10 cc. of lymph produced; $0.10 \times 3 =$ 0.30 cc. total lymph. Dog quiet
3:04 to 3:13 Effect of walking (see table 6)

TABLE 4

Lymph flow and protein concentration during walking

TIME	DISTANCE TRAVELLED	LYMPH FLOW	PROTEIN CONCENTRATION
	meters	*cc.*	*per cent*
1.51			
1.52	100	0.22	1.48
1.53	100	0.12	
1.54	100	0.07	
1.55	100	0.06	1.30
1.56	100	0.05	
1.57	100	0.05	
		0.57	

Flow 0.57 cc. in 6 minutes
$0.95 \times 3 = 2.9$ cc. from foot in 10 minutes

TABLE 5

Lymph flow and protein concentration during running

TIME	DISTANCE TRAVELLED	LYMPH FLOW	PROTEIN CONCENTRATION
	meters	*cc.*	*per cent*
1.57			
1.58	200	0.07	
2.00	200	0.06	
2.01	200	0.05	1.28
2.02	200	0.05	
		0.23	

Flow 0.23 cc. in 5 minutes
$0.23 \times 2 \times 3 = 1.4$ cc. from foot in 10 minutes

TABLE 6

Lymph flow and protein concentration during walking

TIME	DISTANCE TRAVELLED	LYMPH FLOW	PROTEIN CONCENTRATION
	meters	*cc.*	*per cent*
3.04			
3.05	100	0.17	
3.06	100	0.08	
3.07	100	0.09	
3.10	100	0.07	1.34
3.12	100	0.05	
3.13	100	0.05	
		0.51	

Flow 0.51 cc. in 9 minutes
$0.57 \times 3 = 1.7$ cc. from foot in 10 minutes

May 3, 1932. Dog 3. Standing, walking, and increased venous pressure
10:25 Left fore-paw lymphatic cannulated under novocaine
10:30 Lymph protein 0.87 per cent. A few steps

TABLE 7*

Lymph flow and protein concentration during standing and walking and during increased venous pressure, dog 3

TIME	PRESSURE IN CUFF	VENOUS PRESSURE	LYMPH FLOW PER 10 MINUTES	PROTEIN CONCENTRATION	REMARKS
	mm. Hg	*mm. Hg*	*cc.*	*per cent*	
10:50–11:00	0	14	0.07	0.77	Dog sat on haunches and stood
11:00		14		0.77	
11:00–11:10	0	14	0.15	0.67	Walked a few steps and stood
11:10–11:20	0	14	0.10	0.63	Standing only
11:20–11:30	0	14	0.14	0.62	Standing and lying down
11:30–11:40	0	14	0.10	0.57	Standing and lying down
11:40					Blood pressure cuff on and pressure raised to 20 mm. Hg
11:40–11:50	20	26	0.15	0.57	Standing only
11:50–12:00	20	26	0.15	0.52	Standing only
12:00–12:10	20	26	0.23	0.45	Standing. Moved slightly
12:10–12:25	0	14			Cuff pressure released. Dog stood
12:25–12:35	20	26	0.16	0.35	Standing only
12:35–12:45	20	26	0.14	0.33	Standing only
12:45–12:55	20	26	0.36	0.32	Moved slightly. Standing
12:55– 1:05	20	26	0.32	0.29	Moved slightly. Standing
1:05– 1:10	20	26			4 cc. novocaine, adrenin solution infiltrated around wound
1:10– 1:20	20	26	0.70	0.24	Taking a few steps
1:20– 1:30	60	45	0.54	0.21	Standing only
1:30– 1:55					Pressure released. Dog taken for a walk out of doors
1:55– 2:05	40	34	0.29	0.29	Standing quietly
2:05– 2:15	40	34	0.36	0.21	Standing quietly
2:15– 2:25	40	34	0.36	0.21	Standing quietly
2:25– 2:35	40	34	0.49	<0.21	Standing quietly
2:40	60	45			
2:40– 2:50	60	45	0.49	<0.21	Dog lying down and standing
2:50– 3:00	60	45	0.47	0.21	Dog lying down and standing

* All figures for lymph flow in tables 7 and 8 should be multiplied by 3 in order to obtain total lymph from foot.

10:37 Lymph protein 0.77 per cent
10:50 Lymph protein 0.77 per cent. During the preceding 25 minutes the dog stood and occasionally walked a few steps. At 10:50 measurements of the amount of lymph flow began and at 11:40 venous pressure was raised by means of a blood pressure cuff placed around the upper part of the leg (see table 7)

3:10 In order to ascertain the actual venous pressures experienced in this experiment the dog was now anesthetized with nembutal and was placed on a board with legs hanging down: i.e., in the standing position. A large needle was then thrust in a vein in the ankle and the following results obtained:

Normal venous pressure in standing position 14 mm. Hg
Venous pressure with cuff pressure 20 mm. Hg = 26 mm. Hg
Venous pressure with cuff pressure 40 mm. Hg = 34 mm. Hg

TABLE 8*

Lymph flow and protein concentration during passive motion and increased venous pressure, dog 4

TIME	PRESSURE IN CUFF	VENOUS PRESSURE	LYMPH FLOW PER 10 MINUTES	PROTEIN CONCENTRATION	REMARKS
	mm. Hg	*mm. Hg*	*cc.*	*per cent*	
1:05–1:15	0	22	0.42	1.24	Passive motion started 12:45
1:15–1:25	0	22	0.44	1.32	
1:25–1:35	0	22	0.29	1.38	
1:40–1:50	0	22	0.26	1.41	
1:50–2:00	0	22	0.14		
2:20–2:30	0	22	0.17	1.30	
2:30–2:40	0	22	0.14		
3:17	20	22			Raising pressure in cuff to 20 mm.
3:30–3:40	20	22	0.17	1.02	Hg did not increase venous
3:40–3:50	20	22	0.14	1.14	pressure
3:50–4:00	20	22	0.14	1.13	
4:00	40	26			
4:00–4:10	40	26	0.17	1.32	
4:10–4:20	40	26	0.14	1.40	
4:20–4:30	40	26	0.12	1.38	
4:30	70	36			
4:30–4:40	70	36	0.12	1.42	
4:40–4:50	70	36	0.12	1.41	
4:50–5:00	70	36	0.14	1.32	Lymph appears slightly bloody
5:07	100	46			
5:07–5:17	100	46	0.26	1.10	Lymph contains more red cells
5:17–5:27	100	46	0.32	0.97	
5:27–5:37	100	46	0.42	0.85	
5:37–5:47	100	46	0.38	0.78	

Venous pressure with cuff pressure 60 mm. Hg = 45 mm. Hg
Venous pressure with cuff pressure 80 mm. Hg = 64 mm. Hg

May 4, 1932. Dog 4. Complete anesthesia, passive motion and increased venous pressure.

Nembutal anesthesia. Right hind-paw lymphatic cannulated. The dog was placed upon a board with legs hanging down and the leg containing the lymphatic

cannula was attached to a rotating wheel which passively flexed and extended the leg at the knee and ankle joints 20 times a minute. Venous pressures were recorded by means of a cannula tied in a branch of the external saphenous vein (Saphena parva). The progress of the experiment is shown in table 8.

Discussion. It has been our experience, as it has that of others, that lymph does not flow readily from cannulated lymphatic trunks unless the part becomes physiologically active, is massaged or subjected to passive mo-

TABLE 9*

Maximum and minimum amounts of lymph produced during 10 minutes of walking or running in 8 dogs

DOG	MAXIMUM IN 10 MINUTES	MINIMUM IN 10 MINUTES
	cc.	cc.
1	6.7	2.6
2	2.8	1.4
5	1.8	1.4
6	1.2	1.2
7	2.2	1.5
8	0.9	0.9
9	2.4	1.8
10	2.0	0.5

* All figures for lymph flow in tables 9 and 10 have been multiplied by 3 in order to obtain total lymph from the foot.

TABLE 10*

Lymph flow in 3 dogs when flow had become constant during periods of walking or running

NUMBER OF DOG	TIME	ACTIVITY	TOTAL FLOW PER MINUTE
			cc.
2	11:07	Walking	0.12
2	11:33	Running	0.15
2	1:56	Walking	0.15
2	2:01	Running	0.15
2	3:12	Walking	0.15
5	12:04	Walking	0.15
6	11:24	Walking	0.18
6	11:44	Running	0.18

tion, unless the venous pressure is considerably increased so as to increase the capillary pressure, unless the blood proteins are greatly decreased in amount, or unless the blood capillary walls have been so injured as to become abnormally permeable. Furthermore, when observations on lymph flow are made upon different dogs, while the same procedures will cause flow, the amounts of lymph obtained will vary markedly. During the past three years we have dealt with many "dry" dogs, animals from which

nothing save most heroic measures would produce lymph. Save for an impression gained several years ago, and noted by Drinker and Field in 1931, that young animals give more lymph than old ones, we lack any clue to the cause of the differences between individuals. In dogs above 20 kgm. in weight size has been of no moment. All that can be said is that the amount of extracellular fluid which can become lymph apparently varies greatly between animals and in the same animal at different times.

The dogs used in the experiments reported in this paper have produced lymph in fair amounts as soon as the cannula was in place. They represent good "lymph producers." Dog 1 in table 9 is at the high limit of production. The total amounts for 10 minute periods, given in this table, must be a very fair picture of the volume of fluid leaving the blood capillaries during this time. The data in table 10 are, perhaps, even better in attempting to get information upon the amount of blood capillary filtrate during minute periods of steady activity. We have made no attempt to give lymph flow per gram of tissue. While it is probable that most of the lymph collected from the paw arises as a filtrate from the subcutaneous blood capillaries, there are joints, tendon sheaths, skin, and fascial planes about and between muscles and bones. All of these sources are possible contributors to the amounts of lymph we have measured, and certainly all do not contribute equally.

In regard to protein content, lymph produced steadily from the paw of the unanesthetized dog varies from 1.52 per cent (dog 2, table 2, 11.05) to 0.5 per cent (dog 1, 2.10 to 2.35). In six other experiments similar to the first two in this paper, the lymph protein during walking, when reasonable constancy of composition had been reached, lay between 0.69 and 1.34 per cent, with an average of 0.99 per cent. These figures for lymph, steadily produced under normal conditions of activity, are higher than we had expected to find. When the dog stands and moves, the protein concentration in the lymph never increases. It remains constant or decreases. Whenever motion ceases, and the animal lies down, the next lymph collected invariably shows protein concentration. For example, dog 2 at 11:35, following 4 minutes' running, had a lymph protein of 1.42 per cent. At the close of the run he was fed and rested 67 minutes. At the end of this rest lymph could be obtained by massage, and now the protein concentration was 2.12 per cent. Quiescence with lowered capillary pressure and the need for water during digestion, have caused active reabsorption.

When the effects of venous obstruction are followed in table 7, dog 3, an increase in lymph flow and a decrease in protein occur. In table 8, from dog 4, these changes do not take place until the last of the experiment—5.07 to 5.47—when the venous pressure is exceedingly high. This animal was under complete anesthesia (nembutal) against no anesthesia

in dog 3. It has not been our experience that properly given anesthesia affects lymph flow. If it did so there would be an alteration of blood capillary permeability, disastrous for the subject. Differences in degree of effect, such as are seen between dogs 3 and 4, tables 7 and 8, are apparently differences of the moment in the individual animal, causes for which cannot be assigned.

SUMMARY

1. It is a simple matter to cannulate subcutaneous lymphatics draining the hind- and fore-feet of the dog, and to collect lymph for hours as the unanesthetized animal walks or runs about the laboratory.

2. The total amount of lymph produced in the feet of 8 dogs during walking or running, varied between 6.7 and 0.5 cc. in 10 minutes.

3. When activity is uniform, lymph flow becomes uniform.

4. As lymph flow becomes uniform, the concentration of lymph protein does likewise. When constancy of protein was reached in 8 dogs, the concentrations were from 1.52 to 0.5 per cent of protein.

5. Under conditions of steady production of lymph, the lymph collected is held to have the same protein concentration as the blood capillary filtrate and tissue fluid.

6. During rest lymph protein rises rapidly, indicating rapid absorption of water.

7. Graded venous obstruction produces increased amounts of lymph and lowers the protein concentration.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XXIV. The Effect of Insulin on the Blood Sugar Following Total Removal of the Pancreas and Liver

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Mann and Magath, in an investigation of whether the liver is necessary for the hypoglycemic action of insulin, determined the effect of a standard amount of insulin on the blood sugar of a dog before and after the removal of the liver. In order to learn if there was any difference in the rate at which the sugar disappeared from the circulation following the injection of insulin, since both removal of the liver and insulin produce hypoglycemia, it was necessary to administer a large dose of insulin shortly after removal of the liver and before the blood sugar had decreased to any considerable extent due to the loss of hepatic function. They found that insulin produced the same precipitate decrease in blood sugar after removal of the liver as in the normal animal, thus they concluded that the liver is not essential for the hypoglycemic action of insulin.

The only criticism that has been made in regard to these experiments has been that since both removal of the liver and insulin produce hypoglycemia it is difficult to determine that the insulin produced any effect on the dehepatized animal. The precipitate decrease in the blood sugar obtained following the injection of insulin in the dehepatized animal, which was much more precipitous than occurs following removal of the liver alone, would appear to be sufficient answer to the criticism. However, we have been able to demonstrate the action of insulin after the loss of hepatic function in a more striking manner. Although Mann and Magath showed that removal of the liver from a depancreatized animal produces rapid decrease in blood sugar, the sugar is so high in the depancreatized animal before the liver is removed that opportunity is afforded of taking several determinations of blood sugar after removal of the liver and before the administration of insulin, so that it is possible to determine the rate of disappearance of the sugar from the circulation owing to the removal of the liver alone.

The method of experimentation consisted in removing the pancreas from dogs prepared in the usual manner for removal of the liver, and three or four days afterward removing the liver. Determinations of blood sugar

were made before removal of the liver and at half-hour intervals subsequently. Two to three hours after removal of the liver, when the blood sugar was still high, a large dose of insulin was given intravenously and the blood sugar was determined at half-hour intervals. The increased rate of disappearance of sugar from the circulation after administration of insulin was so great that there can be no question the liver is not essential for the hypoglycemic action of insulin (protocol and fig. 1).

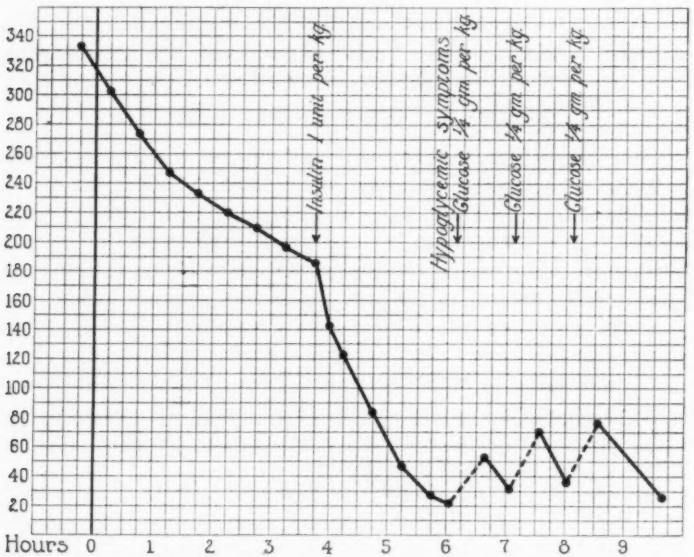

Fig. 1. Decrease in the sugar of the blood following hepatectomy in the depancreatized animal. The abrupt decrease following the administration of insulin may be noted.

REPRESENTATIVE PROTOCOL. October 14, 1931, a reverse Eck fistula was made on a male dog that weighed 11.0 kgm.

January 22, 1932, the dog was in good condition and had increased in weight to 12.2 kgm. The portal vein was ligated.

June 7, 1932, the dog was in excellent condition, weighing 12.6 kgm. The pancreas was removed at 10:20 to 10:55 a.m. The animal was given water but no food by mouth. At 4:00 p.m., 200 cc. of physiologic sodium chloride solution were injected intravenously.

June 8, 1932, at 9:00 a.m., the blood sugar was 218 mgm. for each 100 cc., and 200 cc. saline solution were injected intravenously. At 4:00 p.m. 200 cc. saline solution were injected intravenously.

June 9, 1932, at 8:30 a.m. the blood sugar was 334, and 200 cc. saline solution were injected intravenously. The dog was in good condition after removal of the pancreas

and weighed 11.9 kgm. It was etherized at 8:55 a.m. and the liver, which weighed 300 grams, was removed quickly; the operation was completed at 9:05 a.m. and ether was discontinued.

At 9:15 a.m. the blood sugar was 302. The bladder was emptied. The dog had almost completely recovered from the anesthetic. At 9:45 a.m. the blood sugar was 273. The dog stood and walked and was in excellent condition. At 10:15 a.m. the blood sugar was 248; at 10:45 a.m., 234; at 11:15 am., 200; at 11:45 a.m., 210; at 12:15 p.m., 196.

At 12:45 p.m. the dog was in excellent condition; blood sugar 186, and 12 units of insulin were injected intravenously. At 1:00 p.m. the blood sugar was 142, and at 1:15 p.m., 121. Sixty cubic centimeters of urine were catheterized. Sugar was not present in the urine. The dog was in good condition. At 1:45 p.m. the blood sugar was 80; at 2:15 p.m., it was 46, and the dog was in good condition. At 2:45 p.m. the blood sugar was 27, and the dog was showing symptoms suggestive of the onset of hypoglycemia. At 3:05 p.m. the blood sugar was 22 and the dog was definitely hypoglycemic. Two and eight-tenth grams of glucose (0.25 gm. per kgm.) in 25 cc. of saline solution were injected. Within three minutes the dog was walking and recovery was almost complete; recovery was complete in five minutes.

At 3:40 p.m. the blood sugar was 52; at 4:05 p.m. it was 31, and the dog was in good condition. Two and eight-tenths grams of glucose in 25 cc. of saline solution were injected.

At 4:35 p.m. the blood sugar was 70 and the dog was in good condition. At 5:05 p.m. the blood sugar was 35 and the dog was quiet; it appeared that hypoglycemic symptoms were developing. Two and eight-tenths grams of glucose in 25 cc. of saline solution were injected. Slight recovery had taken place in ten minutes.

At 5:35 p.m. the blood sugar was 76; at 6:40 p.m. the blood sugar was 26 and the dog died.

The peritoneal cavity was almost dry. Nothing was found that would invalidate the results of this experiment.

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STUDIES ON THE INDIRECT MEASUREMENT OF BLOOD PRESSURE

I. Sources of Error in the Riva Rocci Method

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Accuracy in indirect measurement of blood pressure is important, since attempts are still being made to correlate pulse pressure with cardiac output. In spite of this no adequate check of indirect methods has been made. It is true that many comparisons of direct and indirect measurements may be found in the literature, but in the older experiments the direct measurements were themselves open to criticism, and even in the more recent work such as that of Merke and Müller (1925) comparisons were not made during a single pulse cycle, and respiratory or other variations complicate the issue. Petroff (1929) compared indirect measurements on an exteriorised carotid with direct measurements, and found that the indirect estimates might sometimes be too low, but he gives no details of the method employed for direct measurement. The whole subject is further complicated by the necessity of distinguishing between lateral and end pressures, a factor which has been so rightly emphasised by Staehlin and Müller (1925), Merke and Müller (1925) and by von Recklinghausen (1930). Lack of consideration of this factor renders inconclusive the reported lack of correlation between pulse pressure and heart output in experiments such as those of Kisch and Rigler (1930) and of Schoenwald (1931).

Consequently optical methods have been employed both for direct and indirect measurement of blood pressure in dogs, and the system has been arranged so that the indirect estimate can be compared with the directly recorded pressure of the same pulse cycle. In this way sources of error previously not generally recognized have been brought to light, and evidence has been obtained of the magnitude of the total errors.

Methods. Dogs of 12 to 18 kilograms weight have been used. They have been anesthetised with 4 mgm. of morphine sulphate per kilogram injected subcutaneously, followed by an intravenous injection of dial[1] in a dosage of about 0.4 cc. per kilogram. Dial so given acted immediately

[1] We are indebted to the Ciba Company, Inc. for their kindness in supplying dial freely for experimental use. It consists of diallylbarbituric acid, with urethane and monoethyl urea.

(less than 1 minute). If the anesthesia was too light, the experiment was complicated by shivering, tremors or slight movements, and a further dose of dial was given (usually about 1 cc). The anesthesia has proved very satisfactory, has lasted for several hours without further attention; it was supplemented by an additional dose when required. In some experiments anticoagulants were necessary, and in these heparin has been used intravenously in doses of 0.015 to 0.020 gram per kilo.

Comparison has been made in every case between direct records obtained from the right femoral artery 2 to 3 cm. below Poupart's ligament and indirect records obtained simultaneously from the left thigh. Direct records were obtained with Wiggers' (1928) modification of Frank's optical manometer, and his curved cannula of internal diameter 3.5 mm. and length 45 mm. was employed. The rubber membrane of the manometer was heavy (1 mm. or more in thickness) and at one meter distance the record showed changes of 2.5 to 6.5 mm. for 10 mm. Hg actual change in pressure. The constants of the instrument given by Wiggers indicate that with such an instrument no systematic error is to be anticipated in estimations of blood pressure levels; certainly diastolic pressure must be accurately recorded, and even with very abrupt changes early in systole errors are likely to be small. The manometer was standardised several times during an experiment. Corrections were made for the differences in level between the citrate solution and the femoral artery.

In order to measure lateral pressure without interference with the blood flow, the cannula was replaced in some experiments by one of glass, cemented into a brass stem. The glass cannula was T-shaped (fig. 1 B), the stem had a smooth conical junction with the cross piece instead of an abrupt junction of the type pictured by Merke and Müller (1925). The cross piece had a length of 30 mm., an external diameter of 3.5 mm. and internal diameter of 2.5 mm. No rough surface was present at the junction of the T, but the actual cross-sectional area at this point was probably somewhat greater than that of the normal artery. The cross piece was inserted so as to form an integral part of the artery; it was free of constrictions and was carefully cleaned and coated with a thin layer of paraffin wax. Owing to the washing away of citrate by the blood stream, clotting always became evident within 10 to 15 minutes or less, unless heparin had been previously injected. The small dose of heparin combined with the other precautions postponed clotting for an hour or more, but did not completely prevent it. Larger doses of heparin were avoided, for after heparin the pulse rate was often rather fast, and the character of the pulse curve somewhat abnormal, so that there resulted a surprisingly flat pressure curve in the diastolic phase. For instance in one case the lowest pressure in the cycle occurred before and not after the dicrotic wave, for the next pulse followed before this wave had subsided (see fig. 2c and d).

Comparison of lateral and end pressures was attained, with the T-type cannula, by applying temporarily a bull-dog clamp to the artery 2 to 3 mm. distal to the cannula. Similar results could be obtained by obliterating the circulation in the whole thigh by compressing it with a Riva Rocci cuff applied some 4 to 6 cm. below this cannula. The end pressure in the two cases should be recorded with an error due to the elasticity of the vessels between the cannula and the obstruction; in the first case this error should be slight and in the second considerable. No consistent difference was, however, noticeable in the effect produced, but in the second case the collateral circulation was also blocked, which may have compensated for the other variation.

In order to give pulse curves of very different type and to apply a test likely to uncover chance balancing of errors of opposite sign, experimental aortic regurgitation was produced in the animals in the latter part of many experiments, and comparison was made both before and after the production of regurgitation. Injury to the aortic valves was accomplished by the use of stylette introduced down the right carotid artery and forced through one, or more commonly two, aortic cusps (Bazett, 1924).

Indirect measurements of blood pressure on dogs are difficult to make owing to the short conical shape of their thighs and the relatively deep position of the vessles, except at the proximal end in Scarpa's triangle. These difficulties were met with fair success by employing a conically shaped "armlet" of soft pliable leather which somewhat resembled the aluminum cuffs described by Kolls (1920). Slipping of the cuffs down the limb was prevented by anchoring the upper margin to the animal board. The cuffs could be applied snugly with fair ease. Contained within the cuff were usually two rubber bags, the upper commonly some 5 cm. broad and 10 cm. or 20 cm. long, the lower 7.5 cm. or 10 cm. broad and 10 to 15 cm. long. The two bags were usually separated by an interval of about 1 cm., and the total breadth over which pressure was applied was therefore 13.5 to 16 cm., if this intervening small gap be included. However very varying combinations of bags were employed to allow some estimate of the importance of the size of the bags; in some experiments a single bag 12.5 cm. broad and 25 cm. long was employed, and in others double bag systems in which the two bags touched or overlapped. The use of the two bags was copied from the systems described by Gallaverdin (1921) and those used in the Tycos and Boulitte systems.[2]

In dogs of 16 kgm. weight the proximal end of the thigh has a circumference of some 35 cm., the distal of 25 cm., values of similar magnitude to those observed in the upper arm in man. The total breadth of the cuff is

[2] We would like to thank the Taylor Company for their kindness and courtesy in assisting us by supplying us with additional experimental bags, and Dr. D. R. Barr for advice.

therefore one which is comparable to that employed in measurements in man.

The recording system has been a simple segment capsule of 1 cm. diameter, covered with very light rubber dam and contained in a larger

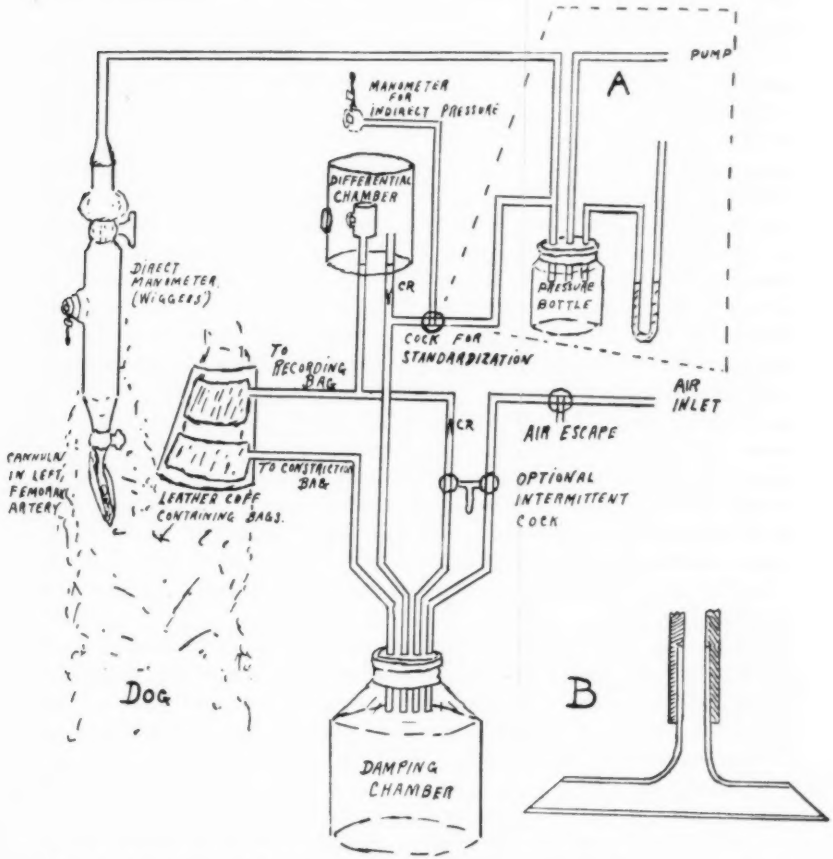


Fig. 1. Arrangement of apparatus for indirect measurement of pressure and comparison with direct readings, except that the limbs used for direct and indirect readings were the reverse of those shown. The part within the dotted line labelled A was used merely for simultaneous standardisation of the two systems. Inset B represents on a larger scale the T-shaped cannula used for direct measurement of lateral pressure.

cylindrical box of 7 cm. length and 6 cm. diameter. The box had a lateral hole with an air tight glass cover to allow records from a mirror attached

to the segment capsule; the bottom of the box was screwed in (with rubber washers) to allow access to the segment capsule, the stem of which was also threaded and attached to the bottom of the box by nuts (with rubber washers) so as to allow adjustment of the mirror in relation to the window. The whole arrangement is illustrated in figure 1. The system as a whole could follow rapid vibrations of 60 per second (e.g., shivering tremors).

The oscillometer was constructed so that it could be used with either continuous or intermittent deflation; either a single or double bag could be used and records taken both during compression and decompression of the armlet. A three-way cock permitted inflation from a compressed air system and subsequent deflation. The air was admitted to the bags and a damping chamber. This damping chamber was connected with the upper bag, the outer part of the differential chamber, and the membrane manometer. The lower bag was connected with the inner part of the differential chamber. Two capillary tube resistances (CR) dampened the pulsations from the upper bag to prevent them affecting either the lower bag or the differential capsule too seriously. A special double cock was also employed, which, when closed, dissociated completely the two bags and kept both sides of the system at constant pressure, except for the pulsations from the vessels. When this cock was left open, the system could be continuously inflated or deflated with such modifications of the pulse form as necessarily occurred despite the damping chamber and the capillary obstructions: by closing this cock from time to time intermittent deflation could be attained. The arrangement used for the simultaneous standardisation of both systems is shown in the upper right corner of figure 1, where it is labelled A. Most of the measurements reported were made with slow continuous deflation.

The oscillometric method was considered the most satisfactory for the purposes of this study because of the relative objectivity of its criteria. Although the Korotkoff sounds can be clearly heard in the dog upon compression of the thigh, their intensity and character are uncertain, so that pressure determinations on a basis of this method cannot be considered reliable. However a few comparisons of different methods were made on man, to permit of greater generalization.

In the use of oscillatory methods, the criteria to be used are still controversial. They are based on two phenomena: changes in size of the oscillations and changes in character of the pulse wave. Considering the former, end systolic pressure is said to coincide with the point, during decompression of the armlet, at which there is an abrupt increase in the extent of the oscillations (Erlanger, 1916a). Lateral systolic pressure according to Müller (1930) is indicated by the pressure level at which the energy value of the oscillations is greatest, i.e., the highest pressure at which the largest oscillations are obtained. The largest pulsations have

been considered by Pachon and Fabre (1924) to indicate mean pressure. Diastolic pressure is generally considered to be the level at which there is an abrupt change from a rapid to a slow decrease in height during decompression (Erlanger, 1916a).

von Recklinghausen (1930) on the other hand has emphasized the value of deductions from the shape and character of the pulse wave, though his records do not appear to be free always from instrumental errors. He has described the changes in wave characteristics as the cuff pressure is altered and has attributed some of these to end pressure, others to lateral pressure effects. The appearance of a dicrotic wave is taken as an indication of the incisura pressure. The diastolic pressure is said to be clearly recognisable since with cuff pressures exceeding this pressure the curves show a flat area at the end of diastole when the vessel is obliterated.

A further criterion for end systolic pressure may be obtained by the use of a double cuff (Gallaverdin, 1921). Above systolic pressure the pulse is blocked by the non-recording upper bag. The first appearance of a recorded positive wave is therefore the pressure level at which the pulse is able to pass the upper bag, i.e., end systolic pressure. All other criteria are presumably similar whether a double or a single cuff be employed.

Results. When comparison was made between direct estimates of end systolic and diastolic pressure and those deduced from the size of the oscillations, it was at once evident that a good check could usually be obtained on end systolic pressure, while the indirect estimate of diastolic pressure was generally much too low. Individual discrepancies in the selection of the criteria existed but even the higher estimates were too low and no definite change in the size of pulsations might be detectable at the true end diastolic pressure level. In the presence of aortic regurgitation these discrepancies in diastolic pressure measurements were much less common. For this reason attention must first be directed to the evidence obtained by direct methods as to the normal differences between end and lateral pressures and the effects of inflation and deflation on these pressure levels, since all of these factors may be involved in causing the discrepancies observed.

End and lateral pressure differences. This difference is a constantly changing figure but at the height of systole it has in three normal animals amounted to from 10 to 27 mm. Hg (mean 18 mm.). At the end of diastole it has varied between − 2 and + 13 mm. Hg (mean value + 4 mm.). After the production of an aortic regurgitation the differences observed in our experiments were 20 to 40 mm. in the systolic phase, and in the diastolic never exceeded 2 or 3 mm. Thus in one experiment (dog I) lateral pressures of 142/92 and end pressures of 152/90 were recorded initially; after production of aortic regurgitation the lateral pressures were 120/64, the end pressures 152/62. The pulse rate increased from 123 to 144. The effect of clamping the artery and changing from lateral pressure to end pressure in two other animals (dogs II and III) is illustrated in figure 2.

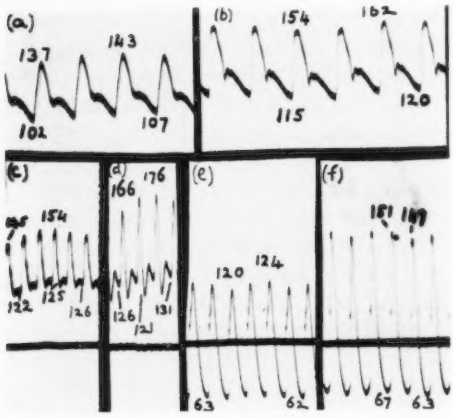


Fig. 2. Comparison of lateral and end pressures. (a) (c) and (e) show lateral, (b) (d) and (f) end pressures. In (b) a clip was applied to the femoral artery beyond the cannula in dog II; in (d) a Riva Rocci armlet was inflated below the cannula in dog III, and (f) shows the effect of the same procedure after production of aortic regurgitation in dog III. Dog III had heparin, dog II none.

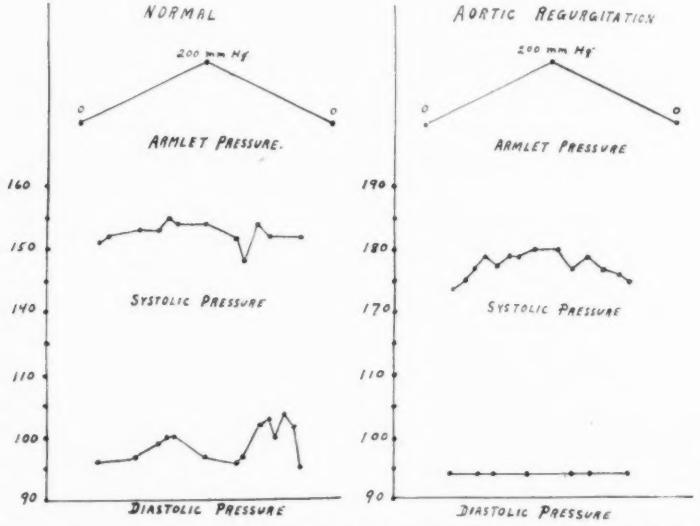


Fig. 3. Pressure changes observed in right femoral artery, when the left thigh is compressed. The uppermost curves indicate the changes in cuff pressure, the other curves the changes in blood pressure.

Effect of compression of the opposite limb with a Riva Rocci cuff on directly recorded pressure. The end pressures recorded directly from the right femoral showed definite but relatively slight changes when a Riva Rocci cuff obstructed the blood flow of the opposite limb (fig. 3, dog IV). Mechanical factors presumably play a part and may account for the greater rise in systolic pressure with inflation in the presence of aortic regurgitation (reflection of a larger pulse wave and consequent increase in the systolic peak in neighboring vessels) and for the fall in pressure noticeable as soon as blood flow into the obstructed limb is renewed. Other slight variations presumably depend on reflex readjustments to the closure of a large vascular bed, adjustments which appear to be less in evidence in the presence of aortic regurgitation. Such reflex changes are attributed by Jansen, Tams, and Achelis (1924) to adjustment within the splanchnic area and are considered by von Recklinghausen (1930) to be an important

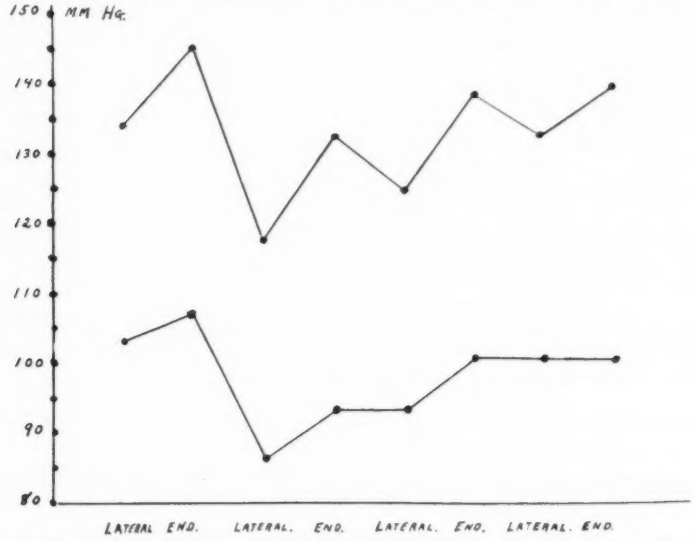


Fig. 4. Lateral pressure changes in right femoral, when a Riva Rocci armlet below the cannula is inflated and deflated repeatedly.

source of error. However in anesthetised animals at any rate they appear of minor importance.

Effects of compression on the pressures in the leg compressed. If the T type of cannula was employed and the thigh was compressed below it by a Riva Rocci cuff, the local changes in lateral pressure in the artery above the cuff during deflation could be analysed.

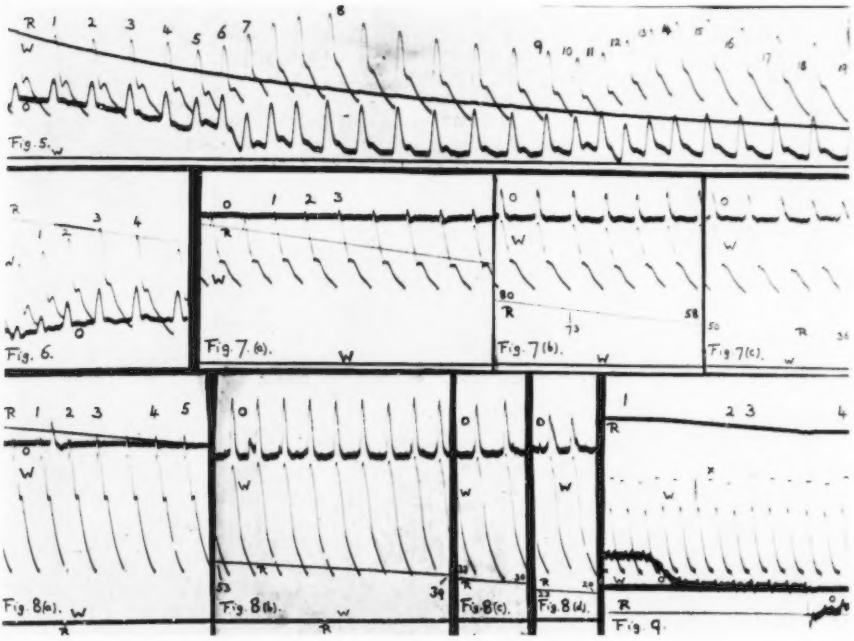
When the Riva Rocci cuff was inflated, the pulse recorded from the T shaped cannula above developed the characteristic changes in shape, exaggeration of the systolic pressure, and increase in pulse pressure always associated with end pressure as contrasted with lateral pressure. The changes in pressure levels observed are shown in figure 4 (dog V). On release of the occluding cuff quite a new phenomenon made its appearance, and this was especially marked if deflation was relatively rapid. This phenomenon was a rapid fall of lateral blood pressures (systolic and diastolic), a

change much greater than that seen on release of a cuff compressing the opposite limb. Thus in the example shown in the figure there was a fall of 17 mm. in both lateral systolic and diastolic pressures. When inflation and deflation was intermittently repeated, these effects became less marked or disappeared. On repetition the systolic end pressure did not in the instance cited attain quite such a high level as at first; this probably depended on some such vasomotor adjustment as that postulated in relation to the effect of cuff distention on the pressure in the opposite limb. The differences between end and lateral pressures became ultimately reduced; this may have been due to the slowing of blood flow through the gradual production of some degree of venous congestion and stasis, for with repeated inflations, the pressure in the bag was probably maintained above the normal venous pressure for some time. Similarly the production of venous congestion and partial stasis may account for the decrease or disappearance of the sudden drop in diastolic pressure on decompression. With the first compression the arteries below the compressing cuff must empty into the capillaries and veins until the pressure in the arteries is quite low; as soon as blood flow recommences a considerable difference in pressure must exist between the columns of blood above and below the cuff and the rate of blood flow must consequently be rapid. The T cannula, and the intervening stretch of artery must act therefore like a Venturimeter; if the velocity is high, and velocity energy therefore considerable, in the absence of any creation of fresh energy, energy in the form of lateral pressure must be correspondingly reduced. With repetition a local slowing of the circulation as the result of congestion may occur but need not imply any great reduction below the normal level, so much as the prevention of the extremely rapid influx of blood of the initial stages of a rapid deflation. It may be noted that throughout the changes illustrated in figure 4, the end pulse pressure was 38 to 39 mm. Hg and the lateral pulse pressure 31 to 32 mm. The congestion was therefore never sufficient to increase the lateral pulse pressure to the end pressure level. Similar adjustments follow repeated application of a clip to the femoral artery; here gradual increase in the collateral circulation may be concerned.

The data quoted indicate that discrepancies in diastolic pressure estimates which do not exceed 13 mm. may be due to comparison of a lateral pressure with an end pressure, but that differences of a greater magnitude cannot with any probability be ascribed solely to this factor; they demonstrate that no comparison of lateral pressures is possible during rapid deflation, since local changes in pressure of a gross kind are induced in the limb submitted to rapid decompression. They also show that reflex adjustments to the obstruction of one limb area do not as a rule produce great changes in pressure and do not introduce major errors; even if present such changes would not invalidate a comparison made during a single pulse cycle. Similarly respiratory variations in pressure, which may be very great particularly if there is also sinus arrhythmia (see fig. 5, dog VI), make comparison of the two methods of measurement more difficult, but do not seriously interfere, when records from each pulse cycle are compared individually.

End systolic pressure. When a single bag system was employed in dogs it was usually impossible to distinguish any definite criterion for systolic pressure in the indirect record on the basis either of height or breadth

of the oscillogram. This may have been due to the transmission of pulsations from the superficial femoral artery at the top of the cuff, where it was only imperfectly subject to pressure. Exceptionally good criteria were obtained in dog VI and yet were not always definite. Parts of two curves are reproduced in figures 5 and 6 and the pressures in the cuff as well as those



Figs. 5 to 9. Simultaneous records by both direct and indirect means. In all of these *W* indicates the record and base line (when reproduced) of the Wiggers' manometer, *R* the pressure and base line indicating the pressure in the cuff, *O* the oscillogram. A description of the cuff systems used is given in tables 1 and 3. The records reproduced in figure 8, (b) (c) and (d), were obtained somewhat earlier after production of aortic regurgitation than was 8 (a). In figure 9 the spiked rises of short duration, *x*, are not readily seen. In a single cycle it has been inked in (with exaggeration of its breadth), and the level reached in the other cycles is indicated by ink marks.

recorded directly (end pressure) for the numbered cycles are given in table 1. The systolic pressure can be estimated fairly readily and accurately in figure 6 and may be determined with accuracy also in figure 5, when analysis is assisted by comparison with direct records. It is, however, unlikely that the point at which the pulse is transmitted would be

read in this record at a single level by different observers. In figures 7a and 8a and table 1 (dog VII) the systolic criteria obtained, when records are made from a double bag arranged as in the Tycos system, are shown. The systolic pressure is indicated very definitely with considerable accuracy and the determination is much less dependent on individual judgment. Lastly in figure 9 and table 1 the criterion is shown as it was demonstrated by a similar system in an animal with aortic regurgitation and with an initial spike-like pressure rise of very brief duration (less than 0.03 sec.). Deflation was here intermittent though records were obtained

TABLE 1

End systolic pressure criteria

FIGURE	PULSE	DIRECT END PRESSURE	BAG PRESSURE	PULSE TRANSMITTED	REMARKS
5	1	140 5	163	−	Dog VI. Single bag 12.5 x 22 cm. Estimated systolic: mean of 5 and 7 = 133.5. Error − 1.5
	2	138	154	−	
	3	138	148	?	
	4	137	142	?	
	5	132 5	137	?	
	6	135	134	? +	
	7	142 5	130	Probable +	
	8	156	122	+	
6	1	161 5	176	−	As in 5. Estimated systolic: mean of 1 and 3 = 172.5. Error of + 1 to + 4
	2	168 5	172	? +	
	3	172	169	+	
	4	170	164	+	
7 (a)	1	140	148	−	Dog VII. Double bag. Upper 5 x 20 cm. Lower 7.5 x 15 cm. Estimated systolic: mean of 1 and 2 = 146. Error of + 5
	2	142	144	+	
	3	143	140	+	
8 (a)	1	171	188	−	Dog VII with aortic regurgitation. Estimated systolic: mean of 1 and 3 = 184.5. Error = + 12
	2	173	184	?	
	3	171	181	? +	
	4	170	175	? +	
	5	169	172	+	
9	1	195	193	−	Dog V. Aortic regurgitation. Double bag. Upper 7.5 x 15 cm. Lower 5 x 20 cm. Peak of 2nd wave 152 to 159 mm. Estimated systolic: mean of 2 and 3 = 186.5. Error of − 5 to − 16
	2	191	187	−	
	3	202	186	+	
	4	203	182	+	

also during the period of deflation. It will be seen that the full pressure of the spiked rise was never indicated and the indirect end systolic pressure estimate was probably at least 10 mm. too low.

The effect of varying the type of cuff is shown in table 2. It seems clear that systolic end pressure may be correctly estimated by very varying

TABLE 2

Type of cuff and error in end systolic estimate

TYPE	SIZE	DOG	RECORD	DIRECT SYSTOLIC	INDIRECT SYSTOLIC	ERROR	AVERAGE ERROR FOR GROUP
	cm.						
Single bag	12.5 x 22	VI	2	160	168	+8	
			3	175	178	+3	+4
			4	173	168	+5	
			5	134	135	−1	
Double bag	7.5 x 25	II	8	140	147	+7	+5
Wide bags	9.0 x 25		12	138	140	+2	
Double bag	7.5 x 25	IV	1	148	154	+6	
Wide upper bag	5 x 20		2	148	157	+9	
			3	152	154	+2	
			5	152	158	+6	+5
			7	152	157	+5	
			10*	182	189	+7	
			11*	178	177	−1	
Double bag	5 x 20	VIII	3	130	134	+4	
Medium upper bag	7.5 x 15		6	127	134	+7	+5
			8	127	130	+3	
Double bag	3.5 x 20	IX	4	130	150	+20	
Upper bag narrow	7.5 x 15		5	125	137	+12	+19
			12	125	150	+25	
Double bag	5 x 10	IX	1	166	189	+23	+24
Armlet loose	7.5 x 11.5		3	192	216	+24	
Double bag	5 x 10		4	176	177	+1	−1
Armlet tight	7.5 x 11.5		8	196	193	−3	

* With aortic regurgitation.

systems with considerable accuracy, but that the estimate is usually somewhat too high. It is essential that the cuff should be applied snugly and that the upper bag should not be too narrow. The upper bag must also not be of too small air capacity. If it is too small, or if the cuff is too loosely applied, pressure is wasted on mere distention of the rubber, while

if the bag is too narrow the pressure is inadequately transmitted to the vessel (von Recklinghausen, 1904). These errors all tend to give too high values, and though they may be reduced to small proportions they cannot be completely avoided. Another error in the same direction may be produced even in a double bag system by transmission of the pulsation of the artery above the cuff through the muscle to the lower bag, even when the vessel is probably not patent. This error may be increased when the pulse pressure is great as in aortic regurgitation (compare errors in figs. 7 and 8). The fact that the total error is usually slight is probably dependent on the partial balancing of these errors by one of the opposite sign. It is improbable that the extreme peak of a spiked-like pressure rise has adequate energy to open the artery along a considerable length against the pressure of the bag, so that the extreme maximum pressure reached is probably not recorded at any rate by a double bag system. Under ordinary conditions this error appears to be small, so that it leaves a slight positive error. When however the pressure change is of very short duration, this error may outbalance the others, and, as in figure 9, give a total error of the opposite sign. Under most conditions the summated error is small, and though considerable total errors are not very uncommon in our series of indirect estimates of end systolic pressure, they have usually depended on chance and not on systematic errors.

Lateral systolic pressure. The criterion of a change in wave character at this pressure level described by von Recklinghausen (1930) was not demonstrable in records obtained on the dog's thigh, and the system used did not allow estimation of the maximum energy such as is required for Müller's (1930) criterion. Consequently no statement can be made as to the accuracy of indirect estimations of this pressure, but it may be stated that at this pressure level no gross change in the character of the pulsation was detected.

End dicrotic wave pressure. The dicrotic wave may appear on the oscillogram at cuff pressures far above the true dicrotic pressure level; thus it may be detected in pulsation *3* of figure 5 at a cuff pressure of some 35 mm. above that of the dicrotic wave. But in this record at lower pressures, between pulsations numbered *7* and *8*, it changed from an elevation of the base line to a distortion of the descending limb of the oscillogram. At pulsation *7* the cuff pressure was some 14 mm. above that of the dicrotic wave, at pulsation *8*, a similar amount below it. An estimate of the dicrotic wave pressure at the cuff pressure at either of the intermediate pulsations would be approximately correct. It is probable therefore that this pressure may be estimable when the character of the oscillographic pulsations is accurately demonstrated. The evidence so far obtained does not allow any more positive statement, nor does it enable one to draw distinctions between end and lateral dicrotic pressures.

Diastolic pressure. It has already been stated that gross discrepancies were found between direct and indirect estimates of diastolic pressure, and some causes for these discrepancies have been uncovered. For if the criterion of decrease in size of oscillation, which is commonly used, represents lateral diastolic pressure it should occur at a pressure somewhat below directly recorded end diastolic pressure, and the mere process of deflation—particularly if conducted rapidly—should also tend to lower locally the pressure that is being measured. But the discrepancies sometimes were even greater than could be accounted for by the sum of these errors, and difficulty in deciding where the rapid decrease in pulsation occurred appeared to be an important factor. Thus in figure 5, when there was a moderate respiratory variation in the size of the pulse, no estimate of diastolic pressure can be made by this criterion. In figure 7 diastolic pressure could not probably be estimated indirectly higher than the second pulse in section (c), or 45 mm., when the real diastolic pressure was 73; it may be noted in 7 (b) that there is a decrease in size of oscillation at this cuff pressure but it certainly is not conspicuous. On the other hand in the same animal after aortic regurgitation this criterion gave an accurate estimate of a lateral diastolic pressure of 30 mm. (last pulse of section c) when the real end pressure was 33 mm. and the true lateral diastolic therefore probably between 30 and 33 mm. Discrepancies (and occasional agreements) of this order have been frequent in our series, so that the criterion cannot be regarded as reliable. It is true that with rapid deflation it can be made much more evident, but under such circumstances the error introduced by rapid deflation more than offsets any advantage gained.

Attention was therefore directed to von Recklinghausen's "formoozsillatorisch" criteria. Occasionally, but only infrequently, the wave characteristics for a diastolic criterion that he described could be demonstrated in our records on dogs; they are certainly much more difficult to obtain from the dog's thigh than from the human arm. A record which exemplifies this criterion is reproduced in figure 5, and though comparison of indirect and direct measurements is hampered by the marked respiratory variation in blood pressure, it will be observed that accurate estimation is possible. In fact wherever this criterion has been in evidence, it has given an accurate estimate of end diastolic pressure. As previously stated this criterion depends on the presence of a flattening of the curve at the end of diastole, when the vessel is completely obstructed, and when no flow is occurring; it must therefore indicate end diastolic pressure. The values of the bag pressures and of directly recorded end pressures for cycles *9* to *19* of figure 5 are given in table 3. Diastolic pressure was estimated at 101.5 mm. at the time of cycles *11* and *12*, when the real values averaged 98.5, at 94.7 at the time of cycles *16* and *17* when the real values averaged 101.0 and at about 92.0 mm. at the time of cycles *18* and *19* when the real

values averaged 94.2 mm. The discrepancies vary therefore between + 3.0 and − 6.3 mm. and are surprisingly small; they depend probably for the most part on the difficulties introduced by the respiratory changes.

TABLE 3

Diastolic criterion in figure 5

PULSE	DIRECT END PRESSURE	BAG PRESSURE	VESSEL COLLAPSED	REMARKS
9	91.5	105	+	Dog VI. Single bag 12.5 x 22 cm.
10	95	103	+	
11	95.5	102.5	+	
12	101.5	100.5	? −	Diastolic estimate 101.5 (mean of 11 and 12)
13	105.5	98.5	−	
14	108.5	98.0	−	
15	105	96	−	
16	102.5	95.5	−	
17	99.5	94	?sl. +	Diastolic estimate 94.8 (mean of 16 and 17)
18	96.0	93	−	
19	92.5	91	sl. +	Diastolic estimate 92 (mean of 18 and 19)

TABLE 4

Comparison of criteria by oscillometric and ausculatory methods on man

(Deflation conducted moderately slowly—about 3 mm. a second)

RECORD	CUFF (DOUBLE)	PRESSURE	SYSTOLIC SOUND	SYSTOLIC SIZE OF OSCILLATIONS	SYSTOLIC APPEARANCE OF OSCILLATIONS	MAXIMUM OSCILLATIONS	DIASTOLIC SOUND	DIASTOLIC SIZE OF OSCILLATIONS	DIASTOLIC CHARACTER OF PULSE
A 1	7.5 x 25; 9 x 25	Inflation	114	112	128	94	74	74	76
A 2	7.5 x 25; 9 x 25	Deflation	110	110	136	92	76	76	76
A 3	7.5 x 25; 9 x 25	Inflation	110	112	—	94	78	78	80
B 1	7.5 x 25; 9 x 25	Inflation	136	143	152	103	—	?	81
B 2	7.5 x 25; 9 x 25	Deflation	138	143	155	103	83	89	98
B 3	7.5 x 25; 9 x 25	Inflation	138	146	152	100	71	?76	77
B 3	7.5 x 25; 9 x 25	Deflation	126	135	145	100	86	88	86
B 4	7.5 x 25; 9 x 25	Inflation	125	132	?137	108	80	80	80
B 4	7.5 x 25; 9 x 25	Deflation	123	134	?144	108	80	80	80
B 5	7.5 x 25; 9 x 25	Inflation	128	146	153	106	97	89	97

The von Recklinghausen criterion seems therefore a reliable indication of end diastolic pressure. Lateral diastolic pressure may be expected to be under most conditions only a few millimeters lower, though under some conditions the difference may exceed 10 mm. When this is the case the

lateral diastolic pressure may probably be estimated by the other criterion of the rapid decrease in the size of the pulsations, if care be taken to locate this point at as high a pressure as possible below the point of the von Recklinghausen criterion.

Mean pressure. According to Pachon and Fabre (1924) the cuff pressure at which the largest pulsations are shown corresponds to the mean pressure. Mean pressure has been estimated in some of our experiments by graphic integration of the directly recorded pressure curve, and this has often corresponded closely with the cuff pressure for maximum oscillations. But there have been many discrepancies and the evidence does not support this method as an accurate one for estimation of either end or lateral mean pressure.

Comparison of various indirect methods in man. Simultaneous measurements have been made in man by the method here described, and by auscultation below the single or double bags employed. In some cases a modified sound capsule was utilised to record the brachial sounds.[3] Some values obtained are shown in table 4.

It will be noted that systolic estimations from an increase in size of oscillations recorded from the lower bag of the system gave values somewhat higher than those estimated by auscultation. These higher values were probably real, since the passage of blood through the cuff at pressures above those at which sounds can be detected, can usually be demonstrated by sensitive methods (Burdick and co-workers, 1925). Erlanger (1916a) considered such a leakage of blood to depend on folds in the vessel wall and thought it no indication of true systolic pressure; it may however represent the passage of the peak of the end pressure wave. On the other hand even with a double bag system positive pulsations may be obtained from the lower bag at much higher pressures. These probably depend on an artifact, since transmission of the pulse from above the cuff through the muscles or upper bag to the lower bag may be recordable with a sensitive system. Consequently even the determination of end systolic pressure by the double bag system may be somewhat dependent on individual judgment.

The diastolic criteria agreed well with one another on the whole. With the moderately slow rate of deflation employed the estimates from a commencing decrease in intensity of sounds, and the lowest pressure at which a large oscillation was obtained agreed well, and these criteria occurred at a pressure a few millimeters below that at which von Recklinghausen's criterion was shown. The change in size of oscillation was however often indefinite and the agreement would probably not be nearly so good if the records of the other criteria had not assisted the observer to reach a decision.

Discussion. The method of comparison employed is probably not open to serious criticism; the pressures in the two femorals were found by Merke and Müller (1925) to be almost identical in dogs (left 2 mm. higher); the

[3] This, designed by one of us (L. B. L.), consisted of a Frank capsule covered with thin condom rubber. At the center was gummed a fine celluloid projection on which one end of the mirror was pivoted. The center of the mirror was balanced on a silk thread extending across the face of the diaphragm, a millimeter in front of it: this thread acted as a fulcrum.

same workers found a slight but definite fall of pressure within the large arteries, so that the pressures in the middle of the left thigh and high up in the right femoral should be practically identical. The manometer used for direct recording has a high natural frequency (certainly above 100 per second in most of the experiments) and the direct readings may be considered absolute.

The importance of distinguishing between end and lateral pressures is clearly indicated, even though the differences observed are less in systole than those reported by Merke and Müller (1925). These workers noted an average difference of 33 mm., as compared with 18 mm. in this series. However the condition of the animals (anesthetic, etc.) was different, for their mean blood pressure values were 179/83, while in this series they were 158/93 without heparin, 148/96 with heparin and after aortic regurgitation 165/56 and 161/52 without and with heparin. A pulse pressure of about 96 gave in Merke and Müller's experiments a difference between end and lateral pressure of 33 mm., one of 109 with aortic regurgitation in this series gave a difference of about the same magnitude, while a pulse pressure of 52 gave one of 18 mm. The figures are therefore not really in disagreement but indicate that the difference between end and lateral pressure in the systolic phase varies with the pulse pressure in almost a linear manner, though it is unlikely that this is the only factor. In Merke and Müller's experiments end diastolic pressures were lower than lateral on the average, in this series higher. The factors involved however are not simple; when flow is prevented any kinetic energy is converted into pressure; this factor will tend to raise diastolic pressure though to a much less extent than end systolic pressure, and it should scarcely function at all in diastole in the presence of aortic regurgitation. This factor would therefore explain the general relationships in our series, but in addition reflection of waves occurs which may exaggerate negative as well as positive waves. This is clearly demonstrated in figure 2 (d), where the pressure level of the notch is lowered from 125 to 121, even though the end systolic is increased 22 mm., and the end diastolic 5 mm., above the lateral pressure levels.

The accuracy obtainable in indirect measurements of systolic end pressure appears to be good, even though any real accuracy depends on errors of opposite sign balancing one another. Provided that the cuffs employed are not too narrow or applied too loosely, the errors appear to be small, unless the pulse curve has an extremely abrupt spike. Under such conditions the indirect estimate may be too low, and the error seems likely to be greater the broader the cuff. Possibly the discrepancies noted by Petroff (1929) had some such cause. In most cases even with a spiked type of pressure rise, it seems probable that the greater part of the spike is of sufficient duration to pass a cuff of the ordinary type and so may be measured by indirect methods, for rarely was too low an estimate made

even in the presence of aortic regurgitation. An instance may be quoted where a brief spiked rise was probably present in man, and was able to pass an ordinary 12 cm. cuff.

A man aged 57 with some arteriosclerosis, a compensated aortic regurgitation and resting blood pressure of 135/35 by auscultation gave after exercise faint but definite sounds below the armlet, even when this was distended with a pressure of 300 mm. The normal systolic criteria appeared at 180 mm. The possibility of extreme effects from water hammer and wave reflection in blood vessels of subnormal distensibility subjected to a very high pulse pressure would appear the most probable cause of such findings.

Cuffs of the size (of a breadth of 12 to 15 cm.) commonly employed in man, as originally recommended by von Recklinghausen, appear to be well suited to the person of average build, since they give a reasonable balance of the opposing errors when employed in dogs with thighs of about the size of a man's arm.

In estimating diastolic pressure the von Recklinghausen criterion dependent on the character of the wave, seems much more reliable than any other. It indicates end diastolic pressure and is not affected by the rate of inflation or deflation of the cuff, except in so far as these give rise to reflex variations in the blood pressure level. Lateral diastolic pressure is normally some few millimeters lower; it may be estimated either by the decrease in the size of the oscillations, or by a commencing fading in the sounds, but the values found must be somewhat dependent on the rate of inflation and deflation. If deflation is too rapid kinetic energy is developed locally from pressure energy, and lateral pressure is lowered; the consistent values obtained by King and Garrey (1928) with rapid deflation were only consistent because they were consistently wrong. With relatively slow deflation the values obtained may be somewhat too high, but the risk of serious error in this direction is much less; moderately slow deflation of two or three millimeters a second is least objectionable.

That the rapid decrease in size of oscillation, when clearly seen, indicates lateral and not end diastolic pressure is evident from its relationship to the von Recklinghausen criterion; for instance, both are clearly shown in one of Erlanger's figures (Erlanger, 1916b, fig. 1), where the difference in cuff pressure between the two points is about 9 mm. Theoretically also no rapid diminution in the size of pulsations should be anticipated as the cuff pressure is lowered from end diastolic to lateral diastolic pressure, for in the former case the vessel is distended by a pulse pressure equal to the difference between lateral systolic and end diastolic pressure and in the latter by one equal to the difference between lateral systolic and lateral diastolic pressure. The distending pulse pressure is therefore slightly greater in the latter case or considerably greater, if the difference between end and lateral diastolic pressures should happen to be great. A rapid diminution in the

size of pulsation is therefore to be anticipated as the pressure in the cuff falls below lateral diastolic pressure.

CONCLUSIONS

A comparison of blood pressure measurements made simultaneously directly on the right femoral artery and indirectly by a modified Riva Rocci method on the left thigh in dogs is described. Both records were made optically. The indirect oscillatory method was demonstrated to be accurate for end systolic pressure whether a single or double bag system was employed, provided that a clear criterion was demonstrable, and that the bags were of proper size and properly applied. A clear criterion was the rule with a double bag system, but in dogs was uncommon with a single bag. A single bag or a double bag system should compress the thigh for a length of at least 12 cm. in dogs of 12 to 18 kgm. weight. In a double cuff system the upper bag must have a breadth of at least 5 cm. Cuffs of similar size are probably suitable for use on the human adult arm. The cuffs must be applied snugly; this can be attained in the dog's thigh by employing a special conically-shaped soft leather cuff, which is anchored to the animal table to prevent slipping.

Changes in blood pressure are produced by inflation of a cuff, partly from mechanical obstruction of a large area, partly through reflex adjustments to this obstruction. These changes rarely exceed 5 to 10 mm. with occlusion of the thigh in the dog; in man with occlusion of one arm the effects are probably less than this.

During rapid deflation, profound local changes may occur in the pressures in the vessels previously compressed. Both lateral systolic and lateral diastolic pressures are reduced, probably as the result of absorption of pressure energy through the induction of a rapid velocity head into the empty vessels below the cuff. In consequence all measurements by indirect means of the lateral pressure levels are too low, unless the deflation is conducted slowly. Moderately slow deflation and consequent congestion does not introduce serious errors. Deflation at a rate of 2 or 3 mm. per second is recommended.

Estimations of end diastolic pressure can be made accurately in dogs when the von Recklinghausen criterion, dependent on the character of the pulsations, can be recognised. In its absence judgments based on the size of pulsation are misleading and usually give rise to estimates of pressure much below the end and probably considerably below the lateral diastolic pressure level.

There is no indication that the largest oscillation indicates either mean end or mean lateral pressure.

Some values for differences between end and lateral pressures in the femoral artery of dogs are given. They were determined by direct methods.

The differences in the two pressures agree only with those reported by Merke and Müller if the magnitude of the pulse pressure is taken into consideration. The systolic difference with a pulse pressure of 52 amounted to on the average 18 mm., in the presence of aortic regurgitation with a pulse pressure of over 100 mm. to one of 20 to 40 mm. In diastole the end pressure might be 2 mm. below or 13 mm. above the lateral pressure; the mean value was 4 mm. above it.

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THE EFFECT OF FEEDING THYROID ON THE SALIVARY CONDITIONED REFLEX INDUCED BY MORPHINE

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Among the general systemic effects of feeding desiccated thyroid substance are a nervous stimulation and tissue dehydration. The work of Kleitman and Crisler (1927) and Crisler (1930) showing the dispensability of the afferent and the efferent pathways respectively, leave the center as the only essential part of the reflex arc for the conditioning process. So far as this paper is concerned, therefore, the important actions of thyroid substance are a stimulation of the cerebrum and tissue dehydration. Stimulation of the cerebrum might be expected to change the tone of reflex centers located there, including the centers which are conditioned by morphine, leading to an increase in the rate of conditioned salivation which could be measured objectively. Tissue dehydration, on the other hand, might be expected to change the tone of these centers in an opposite direction. The present experiments were outlined to determine the resultant effect of large repeated doses of thyroid and thus to test which of these effects is prepotent.

METHODS. A total of eleven dogs was prepared and conditioned with morphine as described by Kleitman and Crisler (1927). After the animals were thoroughly conditioned six daily runs were made for controls. They were then given 2½ to 3 grams of Armour's desiccated thyroid substance daily for the rest of the experimental period (7–18 days) in the hamburg portion of their food. They were kept on their previous maintenance diet and given water *ad lib.*

RESULTS. Before thyroid was started the usual individual variations between animals was apparent, but the customary predictable constancy during different tests in the same animal obtained. After thyroid administration typical signs of experimental thyrotoxicosis developed as especially evidenced by a loss of weight, nervousness, increased food and water consumption and on several occasions muscle tremors. The conditioned secretion of saliva was at first distinctly increased in all dogs. In every case the

[1] These data have been obtained by us independently at various times during the last three years in three separate series of experiments on three different sets of dogs. I have checked the results and combined the materials. G. C.

maximum conditioned secretion of the entire experiment occurred during one of the three daily tests following the first dose of thyroid. From this test on there was progressive, though often interrupted, depression of the secretion. Of the eleven experiments, the maximum secretion occurred on the first day after thyroid (type I) in four, on the second day (type II) in four and on the third day (type III) in three. Figure 1, A, B and C, shows graphs of representative animals of each type. Figure 1 D shows a composite graph of this effect in all dogs plus a composite maximum regardless of when during the three days it occurred. The eleven dogs could also be separated into two classes as to the rate of depression of the reflex (fig. 3). In class I (five dogs) the depressing effect was slow. In class II (six dogs) it was rapid. In general it took 18 days for the decrease in class I to become as pronounced as it did in class II in 7 days. The secretion at the ends of these respective periods showed a decrease of about 40 per cent.

There was no significant correlation between the time of appearance of maximum secretion after thyroid and the intensity of depression. That is, an animal might show type I, II or III stimulating response and be in class I or II. In all but one of the animals in which more than one experiment was run there was a constancy as to type and class. In the one animal, however, there was a switch from one type and class to another type and class.

Feeding thyroid had no detectable effect upon the unconditioned secretion. This is shown graphically in figure 4 where the values before and after thyroid do not vary by more than the daily amounts allowed by the experimental method.

Discussion. The results of these experiments would seem to indicate that both stimulating and depressing effects of thyroid feeding on conditioned salivation are demonstrable though at different times. The first effect of repeated large doses of thyroid is a stimulation of the conditioned secretion presumably from stimulation of the cerebral centers or a lowering of their thresholds. Subsequently the factor of dehydration occurs causing a depression of the reflex. Crisler (1928) showed that dehydration causes inhibition of the salivary conditioned reflex by acting on the central and not on the peripheral nervous system. In the present case thyroid did not affect the unconditioned secretion of saliva so that its action is not on the peripheral system, or, indeed, on any part of the original unconditioned pathway. Barron (1932) likewise found that experimental thyrotoxicosis in the dog does not cause a change in the unconditioned salivary reflex if the animal is given water *ad lib.* It seems probable that the distance effects of thyroid are produced, then, by its dehydrating action. The dehydration in turn causes a depression of the center indirectly.

The grouping of the eleven animals into two classes and of the immediate thyroid effect into three types, probably depends upon the individual make-

up of the dogs. The two classes may correspond to the tendency of a given dog to fall into one of the two classes reported by Kleitman and Crisler (1927) to exist for the extinction of the conditioned reflex in dogs. They found that during unconditioning some animals showed a very rapid rate of extinction while others showed a prolonged rate. The grouping of the animals as to the type of response may mean that dogs giving type I, II or III response are successively more nearly refractory to or have a successively longer latent period for the effects of thyroid substance by mouth.

Zavadovsky (1929) has reported that hyperthyroidation with large single doses in both chickens and dogs leads first to a degradation and removal of the conditioned reflexes in the first days after feeding the thyroid gland and an increase of conditioned indices beginning with the third to fifth days. When fed with small doses of thyroid gland daily, a general increase of the conditioned indices is noticed whereas all the reactions become more exact. These experiments do not parallel ours as we have given large doses repeatedly on successive days. In the third of our animals which showed the maximum stimulatory effect of thyroid on the first day following thyroid feeding, however, our results would appear to disagree with the above, for in such cases a stimulation and not a depression is seen after a single large dose. The differences in our results after a longer time may be caused by the fact that a single large dose of thyroid may cause disproportionately more cerebral stimulation than dehydration initially, but when the feeding is continued there may be disproportionately more tissue dehydration. The differences between large and small daily repeated doses of thyroid may be accounted for by a possibly greater dehydrating effect with the larger doses. In small doses this effect may not be great enough to prevent masking by the tendency of the thyroid to stimulate the cerebrum, but in the larger doses the dehydrating effect becomes too great to be masked and instead becomes prepotent. We are agreed that thyroid feeding (and

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Fig. 1. Conditioned secretion. A, representative type I response (the maximum stimulating effect occurred on the first day after beginning thyroid). B, representative type II response (maximum second day). C, representative type III response (maximum third day). D, composite response of all dogs for three days after beginning thyroid and composite maximum response regardless of when within the three days it occurred.

Fig. 2. Initial stimulating and subsequent depressing effect of thyroid feeding on the salivary conditioned reflex. A, representative class I response (slow thyroid effect). B, representative class II response (rapid thyroid effect). The solid line represents control and the broken line experimental thyroid results.

Fig. 3. Composite curves of conditioned secretion in all dogs. A, class I response (five dogs). B, class II response (six dogs).

Fig. 4. Unconditioned secretion curves before and after thyroid. A, composite curves for all dogs showing a slow thyroid effect (class I). B, composite curve for all dogs showing a rapid thyroid effect (class II).

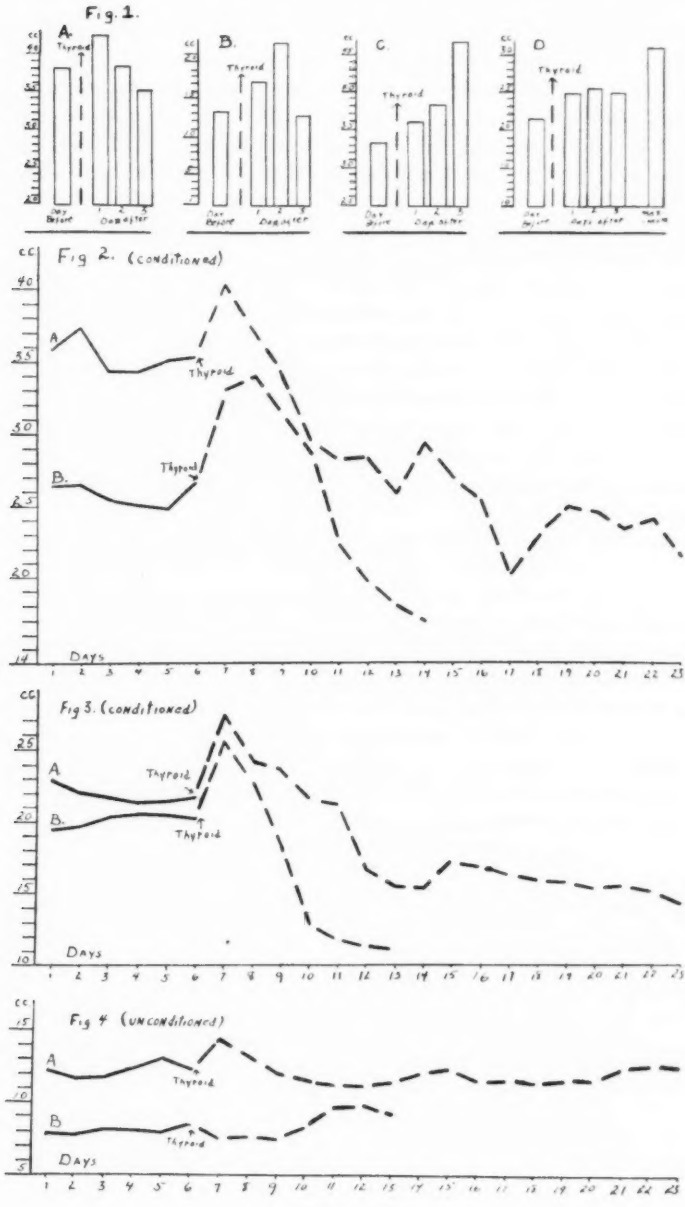
Fig. 1.
A.
B.
C.
D.
Thyroid
Fig 2. (conditioned)
A
B.
Thyroid
Days
Fig 3. (conditioned)
A
B.
Thyroid
Days
Fig 4 (unconditioned)
A
B
Thyroid
Days

Figs. 1–4

Zavadovsky reports the same for thyroid ablation) causes no alteration in the unconditioned secretion rate following morphine injection.

Gulliksen (1931) demonstrated a rise in metabolism accompanying the morphine conditioned state. She ascribed this to the excitement caused by the test conditions during the conditioning process. That the conditioning is associated with and not dependent upon a rise in metabolism is suggested by our results where a further rise in the metabolism is accompanied not by an increased but by a decreased conditioned response.

In our use of the term dehydration in the present paper we do not mean a decrease in the blood volume. We mean a tissue dehydration which leads to a depression of function. As a matter of fact, Rowntree and Brown (1929) in a review of their work and the literature and Wislicki (1930) seem to have shown that there is actually an increased blood volume during hyperthyroidism and a decreased blood volume during hypothyroidism which is attributed to an improper capacity for the physiological storage of blood in these conditions. An inability of the nervous tissue to store blood physiologically would result in a sort of dehydration and lead to a depression of nervous activity.

SUMMARY

1. Large repeated doses of thyroid substance by mouth cause first a stimulation and then a depression of conditioned salivary secretion.

2. The mechanism seems to be an initial cerebral stimulation or sensitization leading to the increased secretory rate, followed by tissue dehydration causing cerebral inhibition resulting in a depression of the secretory rate.

3. Unconditioned salivary secretion is not detectably influenced by thyroid feeding.

4. The conditioned reflex is not accentuated by an artificial raising of the basal metabolic rate by thyroid.

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THE PERMEABILITY OF THE RAT'S PLACENTA TO FAT

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In spite of fairly extensive investigation, the question of the permeability of the placental membrane to fats appears still to lack a definite answer. In reviewing this subject in his comprehensive treatise on *Chemical Embryology*, Needham (1) gives his readers the impression that, although such a conclusion is open to several objections, the bulk of the evidence indicates that fats do not pass through the placenta and, consequently, that the fetal organism is required to synthesize the fatty acids necessary for the elaboration of its essential fatty constituents. Evidence is now available to prove that such a conclusion is contrary to fact. Recently Bickenbach and Rupp (2) have demonstrated that the placenta of the rabbit is permeable to the highly unsaturated fats of linseed oil. In this present paper I am able to offer data which show beyond doubt that the placenta of the white rat is permeable to the highly unsaturated fatty acids present in cod liver oil. It is worthy of note that the placentae of the rat and the rabbit are of the simplest type, only the endothelium of the fetal blood vessels separating the fetal from the maternal blood.

The method which I have employed to determine whether or not fat passes through the placenta of the rat is essentially the time-honored method of determining the iodine number of the fatty acids in the new-born of mothers which had been fed on distinctive fats during the period of gestation. Having found that the phospholipid fatty acids are much more responsive to the presence in the diet of small amounts of unsaturated fats than are those of neutral fat (3), I have determined the iodine number of both the phospholipid and neutral fat fatty acids of new-born rats. The diets of the mothers consisted of commercial casein, cane sugar, salt mixture, dried yeast, and 21.3 per cent, by weight, of either coconut oil or cod liver oil, diets which are known to induce distinctive differences in the iodine numbers of the fatty acids in the tissues of the rat. In order to provide vitamin E, each rat received daily about 5 grams of fresh lettuce. Furthermore each 100 grams of coconut oil contained the unsaponifiable matter from 5 grams of cod liver oil. As indicated in the following table, some of the rats had been raised from weaning age on these distinctive diets; the others were placed on the diets a few days before impregnation.

A day or so before the expected time of delivery the pregnant rat was placed in a special cage consisting of a 12 inch glass funnel with a wire screen bottom in order that, in case the litter should be born during the night, the young would fall through into a padded receptacle without having had a chance to suckle. As will be seen from the table, most of the litters were born during the daytime. Only five of each litter were used. The method used for the extraction and separation of the lipids has been described elsewhere (4). The data are given in the following table.

TABLE 1

Amount and iodine number of lipids of new-born rats

MOTHER RAT		YOUNG RATS		DRY EXT'D. TISSUE PER 100 GM. OF MOIST TISSUE	WEIGHT PER 100 GM. OF MOIST TISSUE			IODINE NUMBER	
No.	Time on diet	Average weight	Approx. age		Phospholipid fatty acids	Unsaponifiable	"Fat" fatty acids	Phospholipid fatty acids	"Fat" fatty acids
Young from mothers on coconut oil diet									
	days	*gm.*	*hours*	*gm.*	*gm.*	*gm.*	*gm.*		
1	140	5.21	<15	11.60	0.726	0.231	0.201	104	69
		5.29	15	11.61	0.726	0.233	0.213	106	69
5	24	4.74	1	11.36	0.701	0.211	0.316	111	59
7	166	5.65	<13	11.20	0.673	0.206	0.276	113	67
1	179	4.94	2	10.85	0.714	0.264	0.351	98	51
7	188	5.49	<10	11.63	0.704	0.227	0.403	104	51
Young from mothers on cod liver oil diet									
2	142	5.11	2	11.60	0.727	0.213	0.249	127	115
3	148	5.53	1	11.43	0.713	0.203	0.304	128	111
4	24	5.44	<9	11.10	0.710	0.208	0.242	124	112
6	26	4.72	1	10.25	0.673	0.185	0.215	125	122
8	>60	2.6*	Prenatal	9.8	0.659	0.197	0.197	122	122
9	>60	2.9*	Prenatal	10.6	0.678	0.188	0.159	120	96

* Entire litters of eight and eleven, respectively, used.

It is quite obvious from these data that the placenta of the rat like that of the dog (Thiemich, 5) and of the rabbit (Bickenbach and Rupp, 2) is permeable to fat. It need scarcely be said that no conclusion can be drawn as to whether the fatty acids pass through the placental membrane as the triglyceride or as a phospholipid.

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SPECTROPHOTOMETRIC COMPARISON OF BLOOD AND MUSCLE OXYHEMOGLOBIN AND CARBOXYHEMOGLOBIN

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The hemoglobin of muscle is so like the hemoglobin of blood that there is a controversy among investigators as to whether they are identical. (For a review of the literature on this subject, see Ray and Paff, 1930.)

Recently Ray and Paff (1930) have presented evidence which indicates that blood and muscle hemoglobin are not identical. They show that the somewhat different absorption curves of blood and muscle hemoglobin are not due to ferment action during the process of extraction (as proposed by Kennedy and Whipple, 1926), because identical curves were obtained from uninjured muscle and muscle extract and because the addition of various hemoglobin decomposition products to blood oxyhemoglobin did not produce a curve characteristic of muscle hemoglobin. Moreover, saturation of muscle hemoglobin with carbon monoxide produced only a 3 $m\mu$ shift in the alpha band, although investigators have invariably found a 6 $m\mu$ shift in carbon monoxide saturated blood pigment.

At about the same time, Dirken and Mook (1930) concluded that blood and muscle hemoglobin are identical. Their findings are based on measurements of the oxygen combining power of muscle hemoglobin ascertained spectrophotometrically. Since this kind of measurement depends upon a predetermined constant (the absorption ratio) and since this constant apparently has not been determined for muscle hemoglobin, it would seem that their claim for the identity of blood and muscle hemoglobin can not be regarded as final.

In order to obtain other evidence for or against the identity of these pigments a different procedure was adopted.

In anticipation of a discussion of such a different procedure, attention is called to the fact that the quotient of the negative log of the transmission at 540 $m\mu$ over that at 560 $m\mu$ is a constant value for any blood pigment in pure solution. This ratio was first introduced by Hüfner (1894) as a means of identifying blood pigment. Various authors have found this quotient to be 1.62 for blood oxyhemoglobin and 1.17 for blood carboxyhemoglobin.

If the same ratio for muscle oxyhemoglobin and carboxyhemoglobin should be found to have different values, there would be additional evidence that muscle pigment is another form of hemoglobin. Experiments were therefore undertaken to establish this ratio for muscle oxyhemoglobin and carboxyhemoglobin.

METHOD. The muscles in the hind leg of a dog were perfused with warm 0.9 per cent saline solution until the fluid which escaped was practically colorless. Ordinarily this was accomplished by forcing the solution slowly through a cannula in the dorsal aorta just above the bifurcation into the iliac arteries and allowing the fluid to escape by way of a cannula in the inferior vena cava at about the level of the aortic cannula. In several experiments the method described by Kennedy and Whipple (1926) was employed. This consisted of repeatedly injecting warm saline solution into the jugular vein alternately with bleeding from the carotid artery.

After removal from the dog the muscle was immediately minced and permitted to stand for an hour or more either in a weak solution of ammonium hydroxide (0.4 per cent) or in distilled water. This fluid was poured off through filter paper (two layers of Whatman no. 5), after which it was ready for spectrophotometric observation. When not in use the muscle hemoglobin solution was kept in an ice box at a temperature near the freezing point. Observations were made by means of a Keuffel and Esser spectrophotometer. Transmission was determined at wave lengths 540 $m\mu$ and 560 $m\mu$ at varying dilutions of the hemoglobin. In no case was the dilution such as to render the readings difficult when either a 1 cm. or a 3 cm. cell was used.

The general procedure was to examine part of each sample in its normal state and part after thorough saturation with carbon monoxide.

DISCUSSION. Each transmission measurement upon which the successive calculations are based is an average of five readings for each determination at each wave length. An average of 47 determinations of the $-\log T_{540}$ to $-\log T_{560}$ ratio gives a value of 1.559 for muscle oxyhemoglobin; and an average of 34 determinations gives a value of 1.354 for muscle carboxyhemoglobin. The standard deviation was found to be 0.052 for oxyhemoglobin and 0.037 for carboxyhemoglobin.

In contrast with the ratio value 1.559 for muscle oxyhemoglobin is the recognized standard value 1.62 for blood oxyhemoglobin. A much more striking difference is noted between muscle and blood carboxyhemoglobin. The average value for muscle carboxyhemoglobin (1.354) as compared with that for blood carboxyhemoglobin (1.17) can not be assigned to breakdown of the hemoglobin, since the value (1.354) is greater than that of methemoglobin (1.22). The interpretation of these results is facilitated by an examination of the complete absorption curves.

Figure 1 illustrates typical absorption curves of blood and muscle oxy-

hemoglobin. The muscle curve represents an average of two transmission readings and the blood curve an average of three transmission readings. Corroborating Ray and Paff (1930) and others, every peak and the dip of the muscle extract curve is 5 $m\mu$ nearer the red end of the spectrum than the corresponding peak and dip of the blood curve. The points 540 $m\mu$ and 560 $m\mu$ do not fall at the peak and depression on the muscle extract curve as they do on the blood curve; hence the lower Hüfner ratio value found for the muscle oxyhemoglobin would be expected. If the ratio of the peak to the depression (i.e., $-\log T_{545}$ over $-\log T_{565}$) is calculated for muscle oxyhemoglobin it is found to be approximately the same as the $-\log T_{540}$ to $-\log T_{560}$ ratio for blood oxyhemoglobin.

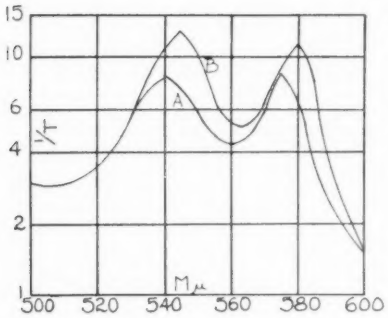

Fig. 1

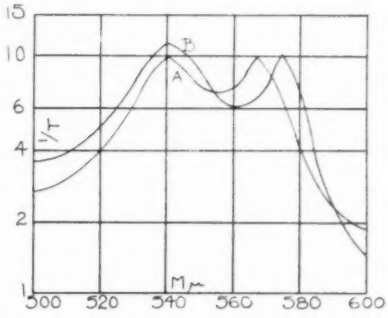

Fig. 2

Fig. 1. Typical absorption curves of blood, *A*, and muscle, *B*, oxyhemoglobin. *T* designates transmission.

Fig. 2. Typical absorption curves of blood, *A*, and muscle, *B*, carboxyhemoglobin. *T* designates transmission.

Typical absorption curves of blood and muscle carboxyhemoglobin are shown in figure 2. In both instances the extracts were completely saturated and well shaken before spectrophotometric observation. Each curve was drawn from an average of two transmission readings. Note that, again confirming Ray and Paff (1930), the first peak in each of the two (blood and muscle extract) curves occurs at 540 $m\mu$, while the depression and the second peak of the muscle curve are each nearer the red end of the spectrum; the depression appearing at 560 mμ, instead of at 555 $m\mu$, and the second peak at 575 $m\mu$, instead of at about 568 mμ. Examination of these curves shows clearly that the ratio of $-\log T_{540}$ to $-\log T_{560}$ would be considerably greater for muscle than for blood. The ratio found in these experiments for blood carboxyhemoglobin is 1.162; this is in agreement with the value 1.168 reported by Ray, Blair and Thomas (1932). On the other hand, for muscle carboxyhemoglobin the ratio cal-

culated from the curve is 1.338, which is in agreement with 1.354 given above.

SUMMARY

While muscle oxyhemoglobin gives a result for its Hüfner ratio value which deviates from that of the blood, the deviation is that which would be expected from the variation of the two absorption curves. The fact that a markedly greater value for the Hüfner ratio has been consistently observed for muscle carboxyhemoglobin than for blood carboxyhemoglobin is clear cut evidence of the non-identity of blood and muscle hemoglobin.

Assistance given in this problem by Dr. George B. Ray is gratefully acknowledged.

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THE RELATION OF CARBOHYDRATES AND LIPIDS IN THE PERFUSED LIVER[1]

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Since the time of V. Mering and Minkowski (1889) a continual search has been conducted for unequivocal evidence of the reversibility of the reaction, carbohydrate → fatty acids. The answer has been sought in three main sources: 1, in the R.Q. in plants and animals; 2, in the D/N ratio in the urine of diabetic dogs and men; 3, in carbohydrate and fatty acid balance experiments in plants, normal and diabetic dogs, perfusions and in pulped tissue of various organs.

Since the liver is generally regarded as the most probable organ for such a transformation, the perfusions of mammalian livers are of particular interest. The perfusion system of Jost (1931) indicates the formation of sugar from lecithin, for upon its introduction into a perfused phlorhizinized liver, the reducing power of the perfusate increased and the R.Q. reached a low level (0.50). These experiments, while suggestive, overlook the possible glycogenolytic action of lecithin (Cruto, 1929). The hazards of R.Q. determinations in such or similar preparations have recently been emphasized by Rapport and Katz (1927). To prove neo-formation of carbohydrate from fatty acids in such a system entails a complete carbohydrate-balance study involving urea, lactic acid, glycogen, free sugar, and fatty acids in both tissue and perfusate, as well as the distribution of these elements. Various workers, Embden (1905), Lattes (1909), and Burn and Marks (1926) have partially fulfilled these conditions. The latter authors report 22 positive perfusions on cat livers freed from glycogen by a fat diet or by depancreatization.

However, these experiments lack quantitative information as to the distribution of carbohydrate from lobe to lobe. (Also no determination of non-sugar reducing substance was made.) Perfusion of such an unphysiological system as an isolated liver, purporting to show carbohydrate formation at the expense of fat, is of questionable validity without these important data. If irregularity of spread should exist, calculation of the carbohydrate balance at the close of a perfusion might very easily result in

[1] Based on a thesis presented in partial fulfillment of the requirements for the degree, Doctor of Philosophy, University of Rochester.

a seeming increase in carbohydrate, whereas actually the opposite might be true. Such experiments, if positive in findings, should demonstrate concurrent lipid changes either in the liver, in the perfusate, or in both. Since none of these necessities appears to have been seriously incorporated into any experiment, it seemed worth while to perfuse a series of livers adopting these precautions.

EXPERIMENTAL PROCEDURE. The experimental procedure was similar in many respects to that of Burn and Marks (1926). The diet, given previous to the perfusions, of the dogs and cats used in these experiments, was in some cases a normal animal-house ration; in others it consisted of 40 per cent cream or hamburg steak (70 per cent fat), fed for periods from 4 to 30 days. In some experiments, the fat diet was supplemented with phlorhizin, adrenalin and starvation, in the attempt to obtain glycogen-free livers.

The technic, as finally worked out for removal of the liver, was very similar to that of Bodo and Marks (1928), although arrived at independently. Heparinized bood (1–2 mgm. per 5 cc. of blood) obtained from a second animal on a stock diet, was placed in the perfusion chamber kept at approximately body temperature. Meanwhile in the fat-fed dog under amytal anesthesia (2 cc. of 5 per cent amytal per kilo) the chest and abdomen were opened under artificial respiration and heparin was injected into the external jugular vein. The pancreatico-duodenal artery was clamped off near its origin, and a cannula inserted peripherally; the hepatic artery was then clamped between its bifurcation and its origin from the coeliac axis, and the first clamp removed. Oxygenated blood from a reservoir was now pumped into the arterial system of the liver through the cannula in the pancreatico-duodenal artery. The portal vein was then cannulated and the animal bled from the carotid artery. The liver was then removed and placed in the perfusion chamber. By means of this procedure there was no interval of time in which oxygenated blood was not flowing through the hepatic artery. The portal flow was interrupted for a very short time (2 min.) for the insertion of a cannula. Very little of the blood from the fat-fed animal was used. In some of the earlier experiments, defibrinated blood of the experimental animal was perfused through the portal vein only.

The perfusion apparatus was enclosed in an incubator kept at approximately 38°C. (fig. 1). The liver was placed on a platform (P.D.) which was connected to a scale (Sc.) reading in grams, from which the weight of the liver at any time could be directly noted. Using a Record syringe as a pump (H.S.) which was connected by an eccentric to a motor, the blood was pumped from the reservoir (Res.) by a double valve perfusion pump (V^1V^2) through the reservoir (Erl.) to the portal vein (Port.) and to the hepatic artery (Hep.) directly, at a total rate of 100 to 200 cc. per minute, the rate depending on the size of the liver used. The perfusion pressure, as read on the mercury manometers, was approximately 15–20 and 50–75 mm. of mercury in the

portal vein and hepatic artery, respectively. The ratio of the blood volume through each was about 3:1. This was regulated by pet cocks and clamps (V³). The blood coming from the vena cava (V.C.) returned by gravity to the perfusion chamber (Res.) after passing through the oxygenating chamber (G.B.). This contained glass beads through which compressed air or oxygen was passed. The ratio of perfusing blood to liver weight varied from 2:1 to 3:1. Perfusions lasted 1 to 3 hours. The perfusion system was allowed to come to equilibrium (5–10 min.), the first 100 cc. of perfusate coming from the vena cava being discarded. Liver samples were then taken from many different lobes. The cut portions were either allowed to bleed, or were clamped off. Blood samples were also removed at this time and the weight of the liver noted. Additional blood samples were removed at convenient intervals

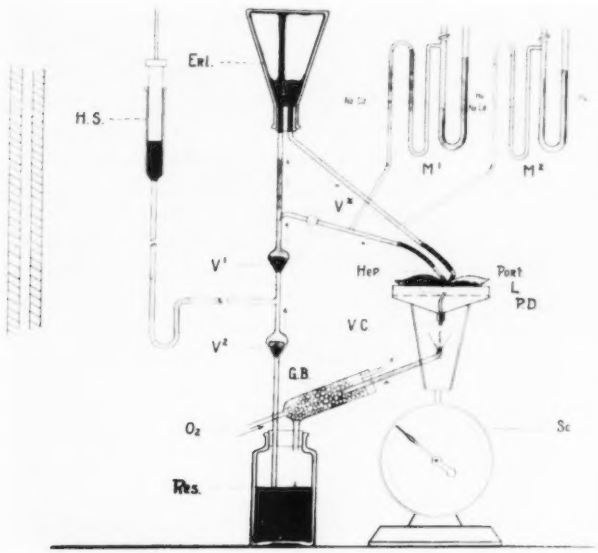

Fig. 1

during the perfusion. Marked edema was invariably observed after 30 minutes unless adrenalin in dilute concentration was added to the perfusate. In perfusion 5, edema was largely eliminated by first circulating the blood through the lungs of the same animal for a half hour previous to the perfusion (Bodo and Marks, 1928). At the close of each perfusion the motor was stopped and the liver allowed to bleed until it came to approximately a constant weight (3–4 min.). This permitted more accurate sampling of the tissue.

The carbohydrate in the blood was determined by the method of Folin (1929). The extraction of free sugar followed the method of Palmer (1917), Burn and Marks (1926); that of glycogen by Büttner's (1926) modification of Pflüger's method. Total carbohydrate was obtained by a modification of Palmer's method (1917). The non-fermentable fraction in both tissue and blood was separated by the method of Somogyi (1928). After extraction of the sugar from the liver it was determined quanti-

tatively by the method of Folin (1929). The total lipids of the liver samples were analyzed according to Kumagawa-Suto (1908) and the blood fats according to Bloor (1928). Blood urea was obtained by the methods of Karr (1924), Meyers (1924), and tissue urea according to Hawk and Bergeim (1927). The method of Friedmann, Cotonio and Shaffer (1927) was utilized for lactic acid.

EXPERIMENTAL RESULTS. *Diet and liver glycogen.* From the work of Burn and Marks one is led to believe that glycogen-free livers are obtained by the simple procedure of feeding 40 per cent cream for four days. This finding in itself appears quite singular in view of what the authors are attempting to prove, namely, carbohydrate formation at the expense of fat. If fats were easily convertible to carbohydrate, it would be more probable that such animals would exhibit a tendency to store carbohydrate derived from fatty acids and that it would be difficult to remove all traces of glycogen by such feeding. In these experiments feeding with cream was noneffective in creating glycogen free livers. Although fat feeding was prolonged more than two weeks and the fat concentration in the liver approached 20 per cent, the glycogen content was in some instances as high as 2 per cent. In only two experiments was it entirely removed. The lowest figures were obtained by supplementing the diet with starvation and phlorhizin (0.077 per cent). The average glycogen content for those animals receiving only cream was 1.27 per cent. This is in accord with the findings of other investigators. Nitzescu and Benetato (1928) found an average of 0.64 per cent in the livers of dogs starved 40 days. Geiger and Schmidt (1928) observed an average value of 0.016 per cent for dogs fasted and phlorhizinized four days. Bodo and Marks (1928) reported normal values for liver glycogen in dogs fed on horse fat. Gregg (1931) found that rats could form glycogen from fat, but not more than could be accounted for by the glycerol-fraction.

Distribution. At the outset it was quite clear that the uniformity or lack of uniformity of the distribution of the tissue constituents in these experiments, namely, glycogen, free sugar and fatty acids, would have a profound effect on the results obtained and upon the interpretation of these results. It was felt that especially in perfusions, in which the organ must of necessity be in a highly abnormal condition, marked variability of the constituents, especially carbohydrate, might occur. Therefore samples were taken from the same and different lobes during the course of a perfusion; some extra livers were also analyzed. These additional livers were analyzed under approximately similar conditions to those used in the perfusions. The findings should be comparable. Table 1 (typical experiments) shows that there was no regularity of distribution of the carbohydrates from lobe to lobe. Analyses on the same lobe also exhibited this uneven spread. In general, the variations were inversely proportional to the sugar concentration. The analyses for "true sugar" do not smooth out this

unevenness for the degree of variation is practically the same as for total reducing substance (table 1). This irregularity does not appear to be greater at the close than at the beginning of the perfusions.

TABLE 1

Distribution of carbohodrate (mgm./gm. of liver)

PERFUSION NUMBER	TOTAL CARBOHYDRATE AND GLYCOGEN					FREE SUGAR					FATTY ACIDS			
	Number of lobes	Number of samples	Total reducing substance	Per cent variation of lobes from average		Number of lobes	Number of samples	Total reducing substance	Per cent variation of lobes from average		Number of lobes	Number of samples	Fatty acids	Per cent variation each lobe from average
				Total reducing substance	True sugar				Total reducing substance	True sugar				
			mgm./gm.					*mgm./gm.*					*mgm./gm.*	
*7B T.CH.	4	8	23.62	±7.7	±9.3	4	8	8.73	±12.0	±12.7	4	8	30.86	±1.2
7E T.CH.	4	8	14.98	±12.8	±21.8	4	8	5.58	±6.3	±4.6	4	8	32.70	±2.2
*16B G.	3	3	21.42	±17.1		3	3	12.20	±4.5		2	2	92.92	±3.0
16E G.	5	6	8.62	±25.2		3	3	13.95	±13.9		2	3	95.89	±7.4
17B G.	3	3	1.48	±7.4		2	2	3.40	±3.3		2	4	68.84	±2.8
*17E G.	4	7	1.08	±8.2		4	4	1.86	±1.1		3	6	49.25	±1.9
18B G.	4	4	21.08	±5.7		3	3	10.12	±11.0		3	6	221.66	±1.4
18E G.	4	6	11.90	±3.5		4	4	10.36	±1.6		3	3	198.60	±1.8
19B G.	4	4	17.28	±13.5		4	4	11.22	±8.4		4	8	208.40	±1.5
19E G.	4	4	7.97	±22.4		4	6	13.47	±3.9		2	4	221.30	±4.9
20B G.	4	8	19.45	±2.2	±0.95	4	8	7.27	±4.5	±7.4	3	6	180.98	±1.5
20E G.	3	6	14.77	±9.1	±12.40	4	8	9.55	±6.3	±5.2	4	8	24.92	±1.2

In experiments 7 and 20 total reducing substance and true sugar were determined; in all the others total reducing substance only.

* B signifies beginning; E end of perfusion. T.Ch. signifies total carbohydrate; G means glycogen.

TABLE 2

Glycogen

LIVER	NUMBER OF SAMPLES	AVERAGE TOTAL REDUCING SUBSTANCE	VARIATION FROM AVERAGE	REMARKS
		mg./gm.	*per cent*	
1	5*	28.62	2.4	Shears
2	7	28.69	5.8	Shears
3	8	17.07	3.9	Liver brei
4	6	12.03	2.7	Liver brei
5	3	13.40	1.4	Liver brei

Owing to the rather large differences obtained in different analyses for glycogen, determinations were carried out to make sure that the method was being properly used. Liver tissue was finely divided with shears or a liver brei mixture made, and the glycogen determined. Table 2 illustrates typical results.

What is the cause of this unevenness in content of the various lobes in carbohydrate? Does it exist in the intact organism, or is it an artificial condition brought about by experimental procedure? Most probably the latter is mainly responsible. During the removal of the liver the scarcity of oxygen no doubt breaks down glycogen in varying amounts from different lobes. In the perfusions themselves the early edema, which is not uniform from lobe to lobe, is a contributing factor. Whatever the cause, the fact itself means that results obtained under such experimental conditions should be interpreted with exceeding caution.

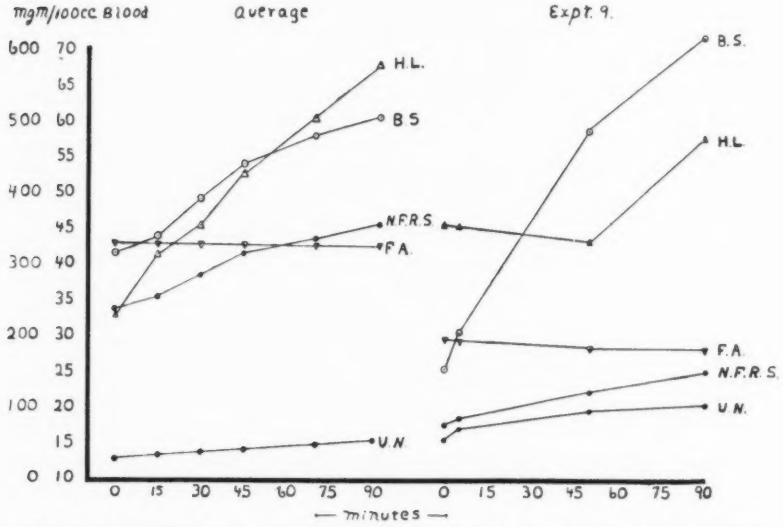

Fig. 2. Blood analyses in a typical perfusion and in the average of all experiments. Ordinates, 0–600 are for blood sugar (B.S.) and for fatty acids (F.A.); 10–70 are for urea and ammonia nitrogen (U.N.), non-fermentable reducing substance (N.F.R.S.) and lactic acid (H.L.).

The liver lipids (table 1), with their even distribution, are in marked contrast to the carbohydrates.

Blood chemistry. The average curves for all the perfusions and also the individual curves for a typical experiment (expt. 9) are exhibited in figure 2. In all the perfusions save one (expt. 8) the blood sugar increased decidedly. In general the blood sugar level was roughly proportional to the initial glycogen content of the liver. That breakdown of the latter was contributing to the formation of the former is attested to by the glycogen analyses at the beginning and at the end of the perfusions.

The small increase of the non-fermentable fraction during the perfusion

is of interest although the cause is obscure. From the work of Barbour (1929) it is possible that it is due to the production of a non-fermentable trisaccharide from glycogen with one-third the reducing power of an equimolar quantity of free sugar. An increased loss of extractives (creatinine, ergothioneine, uric acid and glutathione) from the tissues, due to edema and other unphysiological conditions, may also be suggested as a contributing factor. The rise in urea and ammonia nitrogen indicates a certain amount of deamidization. That sugar is or is not formed from the keto acid residues has not been ascertained in these experiments. Lactic

TABLE 3

Perfusion 4 (true sugar)

	BEGINNING	END
Weight of liver, grams	62.78	55.8
Volume of blood, cc.	420.0	290.0
Blood removed, cc.		134.0
Blood sugar, mgm./100 cc.	153.0	196.0
Free sugar, mgm./gm. liver	3.29	0.895
Glycogen as free sugar, mgm./gm. liver	5.28	3.28
Liver lipids, mgm./gm. liver	75.50	65.65
Blood lipids, mgm./100 cc.	135.00	137.00

TOTAL WEIGHTS	BEGINNING		END	
	Carbohydrate	Lipids	Carbohydrate	Lipids
	gram	*grams*	*gram*	*grams*
Blood sugar	0.644		0.568	
Blood sugar removed			0.246	
Free sugar	0.206		0.050	
Glycogen as free sugar	0.330		0.183	
Liver lipids		4.740		3.670
Blood lipids		0.567		0.400
Blood lipids removed				0.189
Total grams	1.180	5.307	1.047	4.259

acid increased in 7 of 8 experiments. Its source is probably a small glycolysis of blood sugar, especially in those experiments in which the blood sugar was initially at a high level. This contributes to a small loss of carbohydrate at the close of a perfusion.

Perfusions expressed quantitatively. Due to lack of space, the results of only one perfusion (table 3) are presented in detail. This illustrates the type of calculation involved and the character of the results which may be obtained.

Quantitative summaries of total carbohydrate changes during the per-

fusions are given in table 4. Three experiments show an increase in total reducing substance, the rest exhibiting a very definite decrease which reaches in some experiments a rather large figure. Oxidation of sugar by the liver may account for a portion of this sugar loss, although in these experiments this factor can not be quantitated. Lactic acid calculations in a typical experiment (expt. 9, fig. 2) in blood and liver show an increase from 300 to 382 mgm., which accounted for 1 per cent of the carbohydrate dis-

TABLE 4

Carbohydrate and total lipid balance in perfusions

PERFUSION NUMBER	TOTAL REDUCING SUBSTANCE IN GRAMS					TOTAL LIPIDS IN GRAMS		
	Beginning	End	Change	Change (Barbour)	Change as true sugar	Beginning	End	Change
			per cent	*per cent*	*per cent*			*per cent*
2	6.593	4.262	−35.4	−6.7	−31.5	8.549	8.468	−0.9
3	1.440	1.728	+20.0	+23.0		5.575	5.934	+6.4
4	1.180	1.047	−11.3	+0.02		5.307	4.070	−23.0
5	16.620	16.367	−1.5		+0.5	11.695	11.597	−0.6
6	37.400	45.699	+22.0			15.260	14.365	−5.9
7	8.670	5.620	−35.1		−37.1	11.900	9.707	−18.4
8	5.020	4.008	−20.1		−22.5	31.200	36.491	+16.7
9	21.652	13.657	−36.9		−37.4	9.773	8.522	−12.8
11	12.190	7.540	−38.1		−38.7	51.518	37.679	−26.8
12	1.840	1.845	0			121.590	94.808	−22.0
13	1.328	1.045	−21.3		−15.8	115.527	94.845	−17.9
14	4.430	4.654	+5.05			173.245	136.520	−21.0
15	6.508	4.983	−23.4	−6.7		62.767	68.159	+8.6
16	19.073	14.356	−24.7	−1.4		45.654	41.719	−8.6
17	2.783	2.345	−15.7	+4.4		22.638	18.289	−19.2
18	17.868	13.215	−26.0	−7.7		116.103	100.544	−13.4
19	13.622	11.279	−17.2	+3.7		89.421	91.911	+2.70

In perfusions 8 and 9 the diet was hamburg steak (70 per cent fat); in the remaining experiments except 5, 6, and 7, the diet was one pint XXXX cream daily; 12 and 13, in addition to the cream were starved 4 days and also given 10 cc. of 10 per cent phlorhizin (in olive oil) for 4 and 3 days respectively.

* In perfusions 12, 13, 14, 18 and 19 adrenalin hydrochloride was added directly to the perfusion fluid to give a concentration of 1:50,000.

appearing from the system. On the basis of the recent isolation by Barbour (1929) of a polysaccharide with one-third the reducing power of glucose from glycolyzing liver, the results might be recalculated. Such calculations, where applicable, with one exception bring the final total carbohydrate value within experimental error of the method (table 4, column 5). Expressed as true sugar the changes in carbohydrate content of the perfusion are of the same order of magnitude as those expressed as total reducing substance.

Thirteen out of seventeen perfusions were characterized by a decrease in total fatty acid. Since the carbohydrates in such systems also decreased, it is difficult to believe that the lipids disappearing formed sugar.

Discussion. In the foregoing experiments evidence has not been obtained that liver perfusion systems demonstrate a conversion of fatty acids to carbohydrate. On the contrary, the carbohydrate content of these systems generally decreased. Although these results have been given in a quantitative way, it is not believed that they can be accurately measured, since many of the possible factors tending to elevate or to depress the sugar content can not be properly controlled.

The following changes might be conceived to indicate an increase in carbohydrate: 1, an increase in non-fermentable reducing substance; 2, a decrease in lactic acid; 3, protein deamidization; 4, the formation of carbohydrate derived from neutral fat, and 5, the glycolysis of glycogen present in such small amounts as not to be detected by the usual methods for the determination of glycogen. In support of this last point, Evans (1925) found that tissue glycogen in small amounts is quite soluble in the precipitant (66 per cent alcohol) used for its determination. If this non-precipitable glycogen is broken down during a perfusion it will appear as free sugar or blood sugar, giving a pseudo rise in total carbohydrate. All five of the above changes may be determined with some degree of accuracy, but the extent of the contribution of the last three can not be told in such experiments.

All of the factors tending to decrease the sugar content as: 1, lactic acid formation (found to increase somewhat in these experiments); 2, the formation of polysaccharides with lower reducing power (MacCallum); 3, the direct oxidation of carbohydrate by the liver, may be determined accurately.

The changes in carbohydrate content of such systems, exclusive of its possible formation from fatty acids, are, then, a resultant of the above mentioned variables, some lending themselves to determination and, more important, to interpretation, others not. However, even if all these determinations could be adequately interpreted, the distribution of carbohydrate, which was found in these experiments to be very irregular, would largely vitiate any calculations based on these factors.

The above possibilities serve to illustrate the hazards of perfusions. Until all these factors can be adequately controlled, no very positive statements can be made as to neogenesis of sugar from fatty acids.

SUMMARY

1. Fat feeding was not successful in deglycogenizing livers for perfusions.

2. Seventeen perfusions of the livers of fat-fed cats and dogs were performed. These showed *a*, an average decrease in total carbohydrate of

11.9 mgm. per 100 grams of liver per hour; *b*, an average decrease in total lipids of 55.7 mgm. per 100 grams of liver per hour; *c*, a steady production of urea and ammonia nitrogen and of blood sugar, and a much smaller increase in lactic acid and non-fermentable reducing substance; *d*, a marked constancy of the blood fatty acids; *e*, a decided irregularity in the distribution of carbohydrates in such livers, especially those low in carbohydrate.

3. Due to the changes necessarily inherent in such an unphysiological system, namely, lactic acid formation, glycogenolysis, metabolic use of sugar by the liver, edema of the perfused organ associated with marked unevenness of the distribution of sugars, and finally, the ineradicable possibility that sugar may always be coming from glycerol, it is well nigh impossible to use such a system to demonstrate the neogenesis of carbohydrate from fatty acids on a quantitative basis.

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It is a pleasure to acknowledge my indebtedness to Prof. J. R. Murlin, at whose suggestion this work was undertaken.

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THE OXYGEN CONSUMPTION OF RESTING MUSCLE IN RELATION TO PREVIOUS IMMERSION IN RINGER'S FLUID

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Changes in the oxygen consumed by the frog's sartorius muscle after soaking in Ringer's solution have been pointed out by Fenn (1) who found that muscles which had not been immersed in Ringer's before measurement of metabolism used 1.7 times as much oxygen as did muscles which had been so immersed. These untreated muscles were apt to show spontaneous non-irritability and conversely also showed increases in oxygen consumption which were sometimes associated with mechanical shortening of the muscle. Duliere and Horton (2) were able to detect no chemical change in such non-irritable muscles. They found that the phenomena are not due to drying, to changes in the water content, to temperature changes, to previous conditions of the animal nor to gross injury. Horton (3) has, however, found that there is a loss of potassium from the muscle cells when the muscles are soaked in Ringer's. The following experiments were performed to study the changes which might occur in the oxygen consumption of muscles if they were immersed in Ringer's fluid at various intervals after they were removed from the frog.

METHODS. The oxygen consumption was measured by means of a differential volumeter essentially the same as the modified form of the Thunberg microrespirometer described by Fenn (4). Each of the two chambers had a capacity of approximately 10 cc. One centimeter length of the capillary tube corresponded to a capacity of 4.6 cu. mm. of oxygen. The capillary wall was always thoroughly wetted with kerosene before the volumeter was used. The CO_2 was absorbed by filter paper which had been moistened with isotonic NaOH, a film of which also covered the bottom of the chambers. Approximately equal amounts of filter paper and NaOH were placed in each chamber. A sartorius muscle was placed in only one of the chambers but both were flushed out with oxygen saturated with water vapor at the temperature of the thermostat each time the muscle was placed in the chamber. During the experiment the chambers were closed to the atmosphere and in communication with each other through the capillary tubing, separated by the column of kerosene.

The sartorius muscle of the frog, Rana esculanta, was used exclusively in this work. The muscle was carefully dissected out after the tendon had been firmly tied and cut distal to the muscle. A small portion of tendinous tissue at the point of pelvic origin was included in order to fasten the pelvic end. This small amount of extraneous tissue was not sufficient to cause an appreciable error in the amount of oxygen change recorded. The muscle was soaked in Ringer's for 3 to 30 minutes before it was placed in the chamber. After the volumeter had been in the bath for an hour to allow temperature equalization to occur, the readings were started and taken at five minute intervals throughout the experiment. The bath was set at 35°C. and remained constant within 0.005°C. during any one experiment.

Results. The muscles suspended in oxygen in the respirometer in practically all instances presented a gradually increasing oxygen con-

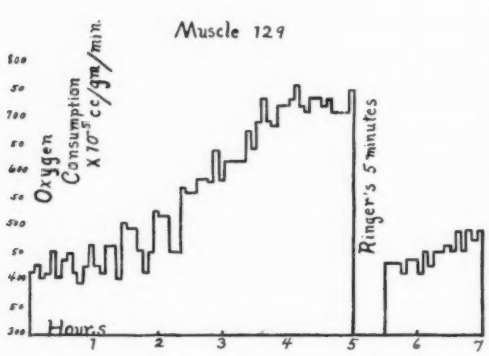

Fig. 1

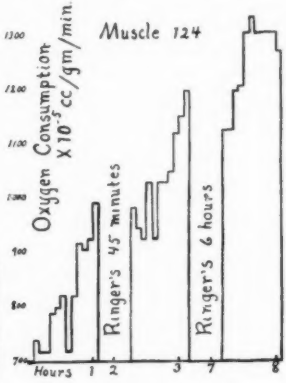

Fig. 2

sumption, as shown in figure 1. After being removed from the chamber, placed in Ringer's for varying periods, and being returned to the chamber, in the typical case the oxygen intake returned to its original level, as in figure 1. There were, however, exceptions to this rule. In muscles 124 (fig. 2) and 321 (table 1), for example, the metabolic rate increased steadily regardless of the treatment. The length of time in Ringer's was not the chief factor in producing this difference because muscle 124 (fig. 2), for instance, was soaked for 45 minutes the first time and six hours the second time. In neither period was there any abrupt change in the metabolic rate, which was high at the beginning of the experiment and continued to increase gradually throughout the experiment.

Muscles 121 and 325 showed a drop in oxygen intake after the first immersion in Ringer's fluid, but the gradual increase in oxygen consumption

thereafter was not interrupted by a subsequent treatment in Ringer's. Muscle 325 returned to a low rate after soaking but increased rapidly, so that, while the average is lower in the half-hour period before soaking than in the half-hour period just following it, there was in reality a definite

TABLE 1

Oxygen consumption $\times 10^{-5}$ *cc. per gram per minute*

HOURS SINCE MUSCLE WAS REMOVED FROM ANIMAL	MUSCLE												
	121	122	123	133	320	321	324	325	430	428a	428b	429a	429b
1.25		316		964						190		264	
1.50		312	320	964		470				190		284	
1.75	848	274	298	964	170	486	436			196		296	
2.00	796	328	374	914	366	546	444	176		200		318	
2.50		344	352	878	610	594	(4)	(4)		230		334	
3.00	720	366	366	806	(2)	630			590	240		398	
3.50	(2)	376	374		470	650				(2)		372	
4.00			392		672	674	486		756			472	
4.50	456	482	418	540	(2)	(4)	544	204	758	140		514	
5.00	494	486	418	520	292		(3)	196	(4)	200		(2)	
5.50	524	430	448	(4)	400			260		210		254	
6.00	560	374	438		(5)	1026		320				392	
6.50	628	338	472	900		1200	440	400	690		210		
7.00	(1)	306	636	890		1194	502	430			220		232
7.50		302				1090	506	452	708		250		330
8.00	852	294				938	438	442	644		240		374
8.50		278						(5)	680				
9.00		296											
9.50		(1)											
10.00													
10.50													
11.00													1020
11.50		746											(2)
12.00	1100	654			360			716					
12.50					520			736					380
13.00													

(1) Immersed in Ringer's fluid for 5 minutes.
(2) Immersed in Ringer's fluid for 15 minutes.
(3) Immersed in Ringer's fluid for 30 minutes.
(4) Immersed in Ringer's fluid for 45 minutes.
(5) Immersed in Ringer's fluid for 3 hours.

drop during the first fifteen minutes of the period following immersion in Ringer's.

Muscles 129, 320, 324, 430, 428a, 429a and 429b showed a drop in oxygen consumption after immersion in Ringer's. Muscle 320 gave this same

response three times. In view of the large variation in the initial rates, and especially because 133, which had been soaked for the shortest time, gave the highest reading, it was thought that the initial period of soaking might not always have been sufficient. Experiments were carried out to control this factor. Muscle 429a was soaked 15 minutes prior to oxygen consumption readings, the control muscle, 429b, was soaked for six hours. The first readings of 429a and the first ones of 429b are practically identical. Approximately the same is true of 428a and 428b, which were treated in the same manner. It would appear that the time of soaking is not the controlling factor.

Discussion. When sartorii show progressive increases in oxygen consumption over a period of time, the rate can frequently be restored to the normal by soaking in Ringer's solution. Since the gaseous intake presented in the table is expressed as the average for half-hour periods the variations in the rate are not as striking as are the original five minute readings given in the graphs.

The length of time, beyond the first few minutes, that the muscles are soaked in Ringer's previous to the beginning of the metabolism measurements appears to be immaterial to the oxygen uptake. All of the muscles with the exception of one were left in the salt solution for at least five minutes, and most of them for longer periods of time, as soon as they were removed from the frog. Since the sartorii used in these experiments were rather small (ranging in weight from 50 to 100 mgm.) five minutes was probably ample time for diffusion equilibrium. In a few instances the increase in the rate of oxygen intake continued regardless of the amount of time of immersion in Ringer's. When muscles are consuming oxygen at a steady rate immersion in Ringer's has little or no effect on their metabolic rate. The average oxygen consumption per gram per minute at 35°C. has been found to be:

NUMBER OF MUSCLES	HOURS AFTER REMOVAL	OXYGEN CONSUMPTION
	hours	*cc. per gm. per min.*
14	1.50	502×10^{-5}
22	1.75	406×10^{-5}
24	2.00	488×10^{-5}
18	2.50	482×10^{-5}

Fenn (5) found a resting oxygen consumption of 255×10^{-5} cc. per gram per minute at 22°C. and 99×10^{-7} at 14.5°C.

CONCLUSIONS

1. Frogs' sartorius muscles, previously soaked in Ringer's for various lengths of time, may show an increase or decrease in oxygen consumption when they are suspended in a chamber of oxygen.

2. Immersing these muscles in Ringer's again usually brings the rate of oxygen consumption back to the original level, although in a few instances the rate is unaffected.

The writer wishes to acknowledge the advice and assistance of Prof. M. B. Visscher during the course of this work.

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THE INFLUENCE OF IODOACETIC ACID POISONING UPON OXYGEN CONSUMPTION OF STRIATED MUSCLE

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Meyerhof and Boyland (1) found that the oxygen consumption of iodoacetic acid poisoned muscle diminished coincident with the loss in ability to produce lactic acid. Hegnauer (2) on the other hand found a recovery oxygen consumption in poisoned muscle which was very little less than in comparable normal muscles. The first mentioned results are to be anticipated if one considers the fact that the oxygen consumption is greatly stimulated by the presence of lactic acid. In otherwise normal muscles Fenn (3) found an increase in the amount of oxygen consumed when they show contracture after treatment with such agents as KCl, NaOH, HCl and caffeine. de Almeida and Martins (4) found a lowered respiratory rate in muscles poisoned with mono-brom-acetic acid. Wright (5) likewise observed a decrease in metabolism following brom-acetic acid poisoning.

In view of the divergent results in the literature it seems desirable to record another set of observations made prior to the publications referred to above, with the method used by Hegnauer, but more closely substantiating the results of Meyerhof and Boyland.

METHODS. The differential volumeter, as described in the previous paper was used for oxygen consumption measurements in these experiments. Sartorii were immersed in Ringer's fluid for several minutes after removal from the frog. The normal oxygen consumption was observed for varying periods of time at 5 minute intervals. After a sufficient number of readings were taken to definitely establish the rate of gaseous intake the muscle was removed and again soaked in Ringer's. The muscle was then returned to the chamber and the oxygen intake again recorded. The muscle was now placed in Ringer's solution which contained iodoacetic acid 1:10,000 for about the same length of time that it had previously been soaked in Ringer's. The muscle was returned to the chamber and its metabolic rate determined in the usual manner.

RESULTS. The usual result of immersion in iodoacetic acid solution has been either a lowering of oxygen consumption below the previous level or no change beyond that due to the salt effect described in the previous paper.

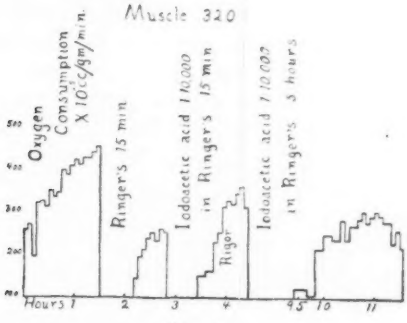
Muscle 320
Oxygen Consumption X 10⁻⁵cc/gm/min.
Ringer's 15 min
Iodoacetic acid 1:10,000 in Ringer's 15 min
Rigor
Iodoacetic acid 1:10,000 in Ringer's 3 hours
Hours 1 2 3 4 5 10 11

Fig. 1

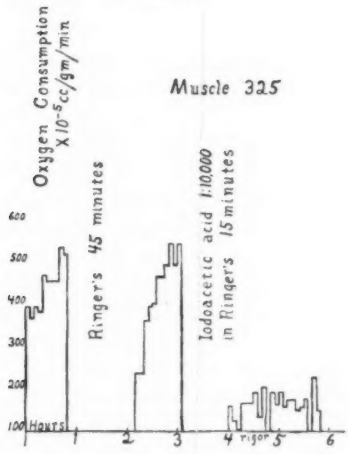
Muscle 325
Oxygen Consumption X 10⁻⁵cc/gm/min
Ringer's 45 minutes
Iodoacetic acid 1:10000 in Ringer's 15 minutes
Hours 1 2 3 4 Rigor 5 6

Fig. 2

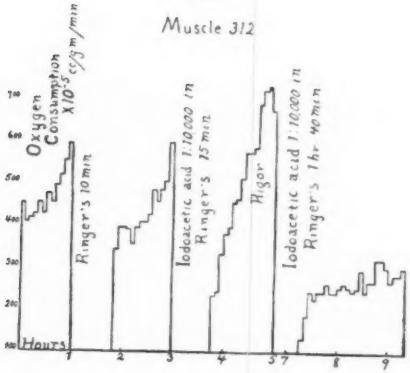
Muscle 312
Oxygen Consumption X10⁻⁵cc/gm/min
Ringer's 10 min
Iodoacetic acid 1:10,000 in Ringer's 15 min
Rigor
Iodoacetic acid 1:10,000 in Ringer's 1hr 40 min
Hours 1 2 3 4 5 7 8 9

Fig. 3

In figure 1 is seen the result of an experiment in which the production of rigor by iodoacetic acid caused no more change in oxygen utilization than the immersion in physiological saline would have produced. In figure 2 the iodoacetic acid treatment was followed by an even lower oxygen usage than followed immersion in Ringer's fluid. Here it appears that the iodoacetic acid had a specific effect in lowering oxygen consumption. Such an effect is not unexpected. The absence of lactic acid production in these poisoned muscles would logically be reflected in a reduction of aerobic restitutive processes, since these are so largely associated with lactic acid removal. Occasionally, as in the experiment of figure 3, there was a questionable increase in oxygen consumption, but in these cases after longer treatment with iodoacetic acid the oxidation rate fell. Smith and Visscher (6) have shown that the concentration of iodoacetic acid determines whether lactic acid production will be stopped or merely slowed. They likewise showed that measurable time is required for iodoacetic acid to affect the enzyme system. It is not unexpected therefore that considerable exposure is necessary to obtain a full effect. It will be noted that the muscle in figure 3 did not go into rigor until the second treatment. The oxygen usage, however, was lower when rigor appeared than it had been previously.

It seems that the deviation of Hegnauer's results from those of Meyerhof and Boyland, and those recorded here, is to be explained on the basis of insufficient duration of treatment and concentration of iodoacetic acid in his experiments. Inspection of his protocols shows that lactic acid production was not entirely inhibited, corroborating such an interpretation.

CONCLUSIONS

Resting muscle treated with mono-iodoacetic acid shows no specific increase in oxygen consumption, regardless of the occurrence or non-occurrence of rigor. A frequent result of iodoacetic acid treatment is a progressive decrease in oxygen consumption.

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PARTIAL SYMPATHECTOMY AND INDUCTION OF PSEUDOPREGNANCY

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Aside from the use of suitable extracts, three methods have been developed for the induction of pseudopregnancy in the experimental animal. Long and Evans (1922) first reported that mating normal females with vasectomized males almost invariably resulted in cessation of the oestrous cycle for a period averaging fourteen days, a period of so-called "pseudopregnancy." These authors further indicated that stimulation of the cervical canals with a small glass rod during appropriate cyclic phases induced a reasonably high percentage of pseudopregnancy responses. Oöphorectomized females bearing ovarian implants also responded, indicating that the phenomenon did not depend upon any possible ovarian nervous connections. Long and Evans' report was subsequently confirmed by Wang (1923), Slonaker (1929), and Meyer, Leonard, and Hisaw (1929). Slonaker, while obtaining uniformly positive reactions to infertile copulation, reported that a considerable number of failures attended mechanical stimulation, approximately thirty per cent, despite the great care exercised. Recently Shelesnyak (1931) has shown that electrical stimulation of the cervix during stages I, II, and III of the oestrous cycle elicited somewhat more satisfactory responses than have been obtained by mechanical means, in that a higher percentage of pseudopregnancies resulted—about 82 per cent, as compared with 70 per cent obtained by Slonaker by mechanical stimulation.

Slonaker expressed the opinion that the factor responsible for the intervention of pseudopregnancy is carried by the blood stream and is a hormonic principle which acts either directly upon the corpora lutea or through some intermediary agent, probably the pituitary. A humoral mechanism, therefore, would be responsible, according to this author, and the hormone would arise in all probability in the vaginal or uterine mucosa.

Meyer, Leonard, and Hisaw (1929), in testing the relative efficacy of mechanical stimulation in anesthetized and in unanesthetized rats, found that anesthesia in itself reduced materially the number of resultant pseudopregnancies. These findings tend to militate against the idea of a humoral mechanism since, as they point out, "if pseudopregnancy is due to sub-

stances released from the mucous membrane of the cervical canals as a result of mechanical injury during copulation or artificial stimulation it seems that this injury would be equally pronounced whether or not the animal is anesthetized." The authors, consequently, are inclined to the belief that a nervous mechanism exercises some control over the mechanical induction of pseudopregnancy.

In view of the foregoing, it is of interest to note Vogt's (1931) recent report that ablation of the superior cervical sympathetic ganglia together with the interganglionic chains resulted in failure of the operated animals to react to mechanical (glassrod) stimulation. Response to mating with vasectomized males, on the other hand, was positive. These results were interpreted to mean that a nervous mechanism is operative in conveying stimuli and that interruption of these nervous pathways, i.e., the sympathetic chains, results in failure of cervical stimuli to reach the pituitary. Moreover, Herren and Haterius (1932) have shown that abdominal sympathectomy, although it has no inhibiting effect upon the oestrous cycle, eliminates the normal cyclic variation in reflex time response—a variation previously shown to be due to ovarian hormonal influences. This would indicate that certain gonadal influences upon the higher nervous levels are effected through sympathetic connections.

In view of the reports cited above, it seemed important that the question of a possible nervous mechanism be subjected to a closer experimental scrutiny. The present report is based upon results obtained in attempts at inducing pseudopregnancy, by the three methods indicated, in animals in which the sympathetic chains had been interrupted. In one series of animals this interruption was effected by abdominal sympathectomy; in a second series recourse was had to ablation of the superior cervical sympathetic ganglia.

EXPERIMENTAL METHODS. Thirty sexually mature female rats, averaging 195 grams, were selected as experimental animals, the selection being based upon the regularity of their oestrous cycles as determined by daily vaginal smears for one month prior to operation. Bilateral abdominal sympathectomy was performed upon eighteen of these, using intraperitoneal injections of Pentobarbital sodium ("Nembutal") for anesthesia. Under a dissecting microscope and with aseptic precautions the sympathetic chains were removed from the level of the renal veins through the pelvis, including in removal the parasympathetic pelvic nerves. Muscle layers and skin were sutured separately with silk and after a recovery period of three weeks the oestrous cycles again were determined. In all but one instance their regularity was uninterrupted. Seventeen operated cases, therefore, were available for experiment.

In the remaining twelve animals, the superior cervical sympathetic ganglia were removed under the same routine employed in the first series.

Cycles, after recovery, continued in their regularity. Subsequently, however, two of these animals became sick, and were destroyed.

Twenty-four control animals, running regular cycles, were subjected to the same subsequent treatment as the experimental animals. Cycles in all instances were followed daily.

In attempting to induce pseudopregnancy, three methods were utilized: 1, by mating with vasectomized males; 2, by glass rod stimulation of the cervical canals, and 3, by electrical stimulation of the cervix. Results obtained will be discussed in order.

1. Infertile copulation. Upon full recovery, oestrous cycles were followed daily, and, as each animal came into late pro-oestrus (mixed nucleated and cornified cell stage), it was placed in a large cage with a number of vasectomized males. Copulation in nearly every instance was observed directly, and in all cases was verified by discovery of a vaginal plug a few hours subsequently. If copulation failed to occur at any given exposure to males (a period usually of twelve hours), the routine was repeated at the next pro-oestrous stage, and so on, so that in time all animals, including experimental ones and controls, had been successfully mated.

TABLE 1

Effect of sympathectomy upon induction of pseudopregnancy

ANIMALS; TYPE OF OPERATION	NUMBER OF CASES	NUMBER OF RESPONSES	NUMBER OF FAILURES	PER CENT RESPONSE
A. By infertile copulation				
Abdominal sympathectomy	17	17	0	100
Cervical ganglionectomy	10	10	0	100
Unoperated controls	24	23	1	95.8
B. By mechanical stimulation				
Abdominal sympathectomy	16	0	16	00.0
Cervical ganglionectomy	10	0	10	00.0
Unoperated controls	24	11	13	46.8
C. By electrical stimulation				
Abdominal sympathectomy	16	1	15	4.3
Cervical ganglionectomy	10	0	10	00.0
Unoperated controls	24	22	2	91.6

Results obtained by this procedure are tabulated in table 1, section A, and show that, with one exception, pseudopregnancy was induced in all instances. The one failure was a normal control which did not respond although definite mating occurred on two occasions; this rat, however, subsequently responded to electrical stimulation. The pseudopregnant

period averaged twelve days. Oestrus almost invariably occurred on the thirteenth day following copulation although in a few instances the dioestrous period persisted thirteen or fourteen days. Operative removal of portions of the sympathetic chains, therefore, irrespective of whether cervical or abdominal, had no effect whatever in inhibiting the induction of pseudopregnancy as a result of infertile copulation.

One of the animals in the abdominal sympathectomy series displayed a continuous oestrous smear subsequent to pseudopregnancy and was discarded.

2. Mechanical stimulation. Upon resumption of the oestrous cycle following the pseudopregnancy induced by infertile mating, each animal, during a late pro-oestrous phase, was subjected to mechanical stimulation of the cervical canals, for which purpose a small glass rod was employed. In the first trial, all animals were placed under ether anesthesia; subsequent attempts were made without anesthetic. Table 1, section B, indicates the results obtained in this experiment. It will be noted that in no instance was pseudopregnancy induced in an experimental animal. On the other hand, positive response on the part of controls was very unsatisfactory, and a percentage was obtained (46.8) considerably lower than that reported by previous investigators. Repeated attempts were made upon each animal, as each pro-oestrous phase appeared, but as many as six or eight attempts failed to induce a response in experimental animals. Success was occasionally achieved in controls, however, when repeated stimulation was tried. In many instances cotton plugs were inserted in the vaginal canal following stimulation, on the possibility that a plug might facilitate a reaction, but in no instance did this prove to be the case. Stimulation during oestrus as well as during pro-oestrus was without result.

In view of the large number of negative responses in control animals a reasonable doubt might arise as to the efficacy of the technique employed. However, the greatest care was exercised, glass rods of varying calibre were used, and frequently stimulation was prolonged to a point beyond which it was feared undue irritation might ensue. It might be argued that a colony difference could account for the disparity between present results and those recorded elsewhere, but this rather trite alibi seems inadequate, and there is nothing on record to date to indicate that such differences may exist with respect to the comparative ease of stimulation. Suffice it to say, results obtained by the writer through the use of mechanical stimulation, while wholly negative in operated animals, are rendered somewhat inconclusive because of the very great difficulty encountered in arousing a response in the normal control.

3. Electrical stimulation. Following rather exhaustive attempts at successful mechanical stimulation, the entire group was subjected to elec-

trical stimulation of the cervix. For this purpose apparatus somewhat similar to that described by Shelesnyak was employed, consisting in a two volt storage battery, a resistance coil, and electrodes of fine Nichrome wire, insulated to the tips and the latter spaced a millimeter apart. During the early pro-oestrous stage each animal was given a mild electrical shock, with the aid of a speculum, directly upon the cervix. Eight animals of the experimental and eight of the control group were stimulated under deep ether anesthesia. No response was elicited in either group. Following this, the entire group was stimulated without anesthetic. Results are arranged in table 1, section C, which demonstrate that the method of electrical stimulation is highly satisfactory for inducing pseudopregnancy in the normal animal. Reactions of the operated animals, however, with one exception were uniformly negative, despite repeated attempts and, in many instances, quite strong shocks. The high percentage of response in controls, as listed, is due partly to the fact that when the first attempt failed, stimulation was repeated at the next pro-oestrus; in only two instances was a third stimulation necessary. Two normal animals failed entirely to respond. The same procedure, of course, was followed in the experimental group as well, some animals receiving as many as eight stimulations in as many cycles.

These results appear definite enough to warrant the conclusion that sympathectomy, whether cervical or abdominal, inhibits successfully the induction of pseudopregnancy by electrical means, and it seems probable that the failure to secure a positive response with mechanical methods may not be disregarded, despite the surprisingly low response on the part of controls in that instance.

Several of the sympathectomized animals subsequently were allowed to mate with fertile males. Pregnancy ensued, normal delivery occurred, and the litters were reared in normal fashion. Bacq (1932) reported a similar behavior in his sympathectomized rats.

Upon completion of the work all experimental animals were sacrificed, autopsied, and the operation was verified in all instances.

Discussion. The observation that interruption of nervous pathways prevents the induction of pseudopregnancy either by electrical or by mechanical means appears to coincide with the results reported by Meyer, Leonard, and Hisaw since, if a humoral mechanism were operative it would not seem likely that resection of sympathetic chains or removal of the superior cervical ganglia would affect materially the induction of pseudopregnancy.

The nervous pathway apparently is a sympathetic one. The pituitary itself, an internally secreting gland, would be expected to have an autonomic innervation. Stimuli hence would reach the gland by means of the superior cervical ganglia. Such a view is substantiated by Dandy's (1913)

demonstration of an autonomic innervation of the pituitary in the dog, in which he reported that nerve fibers from the carotid plexus supply both lobes of the hypophysis. Pines (1925) has more recently advanced evidence in support of these earlier findings. It is not improbable, therefore, that the hormonal release in the pituitary which presumably initiates the condition of pseudopregnancy depends normally upon stimuli originating from irritation of the cervical canals and traversing the sympathetic chains to the pituitary. Anesthesia would tend to block the passageway; operative interruption would be equally effective.

The fact that operated animals respond to infertile copulation—an observation first noted by Vogt—does not necessarily argue against a nervous mechanism. Vogt believes that the vaginal plug acts as an aid in stimulation in this circumstance. In the present work cotton plugs, fitting tightly into the vagina, were inserted in many instances following mechanical stimulation and allowed to remain for about twelve hours, on the assumption that they would serve as effective substitutes for the vaginal plug. No difference was noted, however, so far as results were concerned. It seems more probable that the "psychic" factor (i.e., sexual excitement), influential in the act of mating but undoubtedly submerged during artificial stimulation and certainly so during anesthesia, might well act as a stimulus sufficient in itself. It is well known, for example, that in the rabbit, a non-spontaneously ovulating form, ovulation frequently results from the stimulus of great excitement, proximity of males or, not uncommonly, from association with other does.

It is noteworthy, in the present connection, that interruption of the sympathetic chains inhibits the influence of ovarian hormones upon certain higher nervous levels, particularly with regard to the control these levels exercise over reflex time response (Herren and Haterius, 1932). Thus it appears that the integrity of the nervous mechanism is essential, not only for the passing of stimuli from the genital region to the pituitary, but for the physiological efficacy of ovarian principles in relation to higher nerve centers as well.

SUMMARY

1. In experiments devised to ascertain the effect of interruption of the sympathetic chains upon the induction of pseudopregnancy, two series of rats were employed; one in which lower abdominal sympathectomy had been performed, a second in which the superior cervical sympathetic ganglia had been removed.

2. Response to infertile copulation was uniformly positive, indicating that nervous mechanisms are not necessary in this circumstance.

3. Response to mechanical stimulation was negative despite continued and repeated stimulation. Response on the part of normal controls was poor, averaging 46.8 per cent.

4. Electrical stimulation was uniformly negative in the experimental animals. In normal controls, on the other hand, pseudopregnancy almost invariably ensued.

5. The fact that operated animals respond to infertile copulation may perhaps be explained on the basis of the factor of sexual excitement. Such a factor would be absent under experimental conditions.

6. It is concluded that a nervous mechanism is operative in the artificial induction of pseudopregnancy in the rat. Nervous connections, on the basis of present and of previous anatomical and experimental evidence, appear to lie in the sympathetic chains.

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THE HEART AND MEDIAN CARDIAC NERVE OF LIMULUS POLYPHEMUS

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The utilization of recent developments in the amplification and recording of electrical potentials by thermionic vacuum tubes has made possible certain observations on the heart and median cardiac nerve of Limulus polyphemus which throw light on the nature of its heart contraction and the nerve activity associated with it. An analysis of the electroneurograms of the median cardiac nerve has made possible a correlation between the potential record and the histological structure of the nerve cord. Direct observations have been made of the manner of action of extrinsic nerve fibers on intrinsic cardiac ganglion cells. The classical studies of Carlson, (1909), Hoffmann (1911), and Nukada (1918) indicated quite definitely that in Limulus the origin of the heart beat was neurogenic. The present report gives objective proof to their deductions. The electrocardiographic studies show that the heart contraction is a tetanus. Two preliminary reports by the author, (1930) and (1931), have appeared on this subject.

MATERIALS AND METHOD. Limuli were obtained from the Marine Biological Laboratory at Woods Hole. They were shipped to St. Louis, packed in excelsior, moistened in sea water. On arrival they were kept in a cold room until used. The animals shipped and kept in this way were quite satisfactory for our purpose.

The heart and median cardiac nerve were studied both *in vivo* and *in vitro*. Auricular and ventricular myograms were recorded using a light spring heart lever. The attachment to the heart structures was effected by very light metal hooks to avoid trauma and blood loss to as great a degree as possible. The kymograph used was capable of a speed up to 150 mm. per second. The electromyograms and electroneurograms were made with an amplifier-oscillograph combination of a type described in previous papers from this laboratory. The amplifier yielded amplification up to 1200 mm. per millivolt. A camera device for the taking of continuous records was utilized. Records of both autochthonous and artificially induced activity were made. For electrical stimuli condenser

charges of the required capacity and voltage served. Absolutely refractory period determinations were obtained by a method previously described. For chronaxie determination galvanic current was applied through small hooked silver electrodes with a resistance of 10,000 ohms placed in the stimulating circuit. These were placed about 1 cm. apart and the area kept free from fluid. In the beating heart all readings for chronaxie determinations were taken during the last third of the interventricular pause. The duration of the currents used was measured on the oscillograph.

Experimental findings. In view of the recent failure of Dubisson (1931) to support the older view of Carlson as to the effect on the ventricle of removal of the median nerve cord, experiments were first made to determine whether the removal of the median cardiac nerve resulted in a temporary or a permanent cessation of ventricular activity. It was found that if the normal distention of the ventricle was maintained by air or oxygen the beat would spontaneously reëstablish itself after fifteen to thirty minutes. Once the beat was reëstablished it was not always necessary to continue the distention in order to have the activity continued. In a good preparation it was possible to cut the heart into transverse segments as short as one and one-half centimeters and have the segments continue to beat. Such segments could be obtained from all parts of the heart including the first three anatomical segments where it is known that the median nerve cord contains very few ganglion cells. In many of the preparations studied special care was taken to dissect off the radiating branches of the median cardiac nerve because these are known to contain a few ganglion cells. Two of the $1\frac{1}{2}$ cm. long active segments just referred to were cut into five micron serial sections and on examination failed to reveal the presence of any ganglion cells. It is considered, therefore, that the heart muscle of Limulus is in itself potentially rhythmic.

Experiments to determine the nature of the contraction in the normal ventricle indicated that the beat occurred practically simultaneously in all its parts. The type of spontaneous activity in the ventricle freed of the median cardiac nerve was easily distinguished from the type of activity in the ventricle normally innervated. In the former preparation the activity is propagated from some variable point of origin by a slow peristalsis, the conduction rate of which is 1 to 2 cm. per second. Frequently the point of origin is in the first two segments. In the normally innervated heart the fourth and fifth segments have been found to possess the highest degree of spontaneous rhythmicity and therefore the region of the median cardiac ganglion corresponding to this may be regarded as the pacemaker. In good deganglionated preparations the origin of spontaneous activity may become quite diffuse and the type of beat approximate the normal. It is evident that the type of activity in the distended

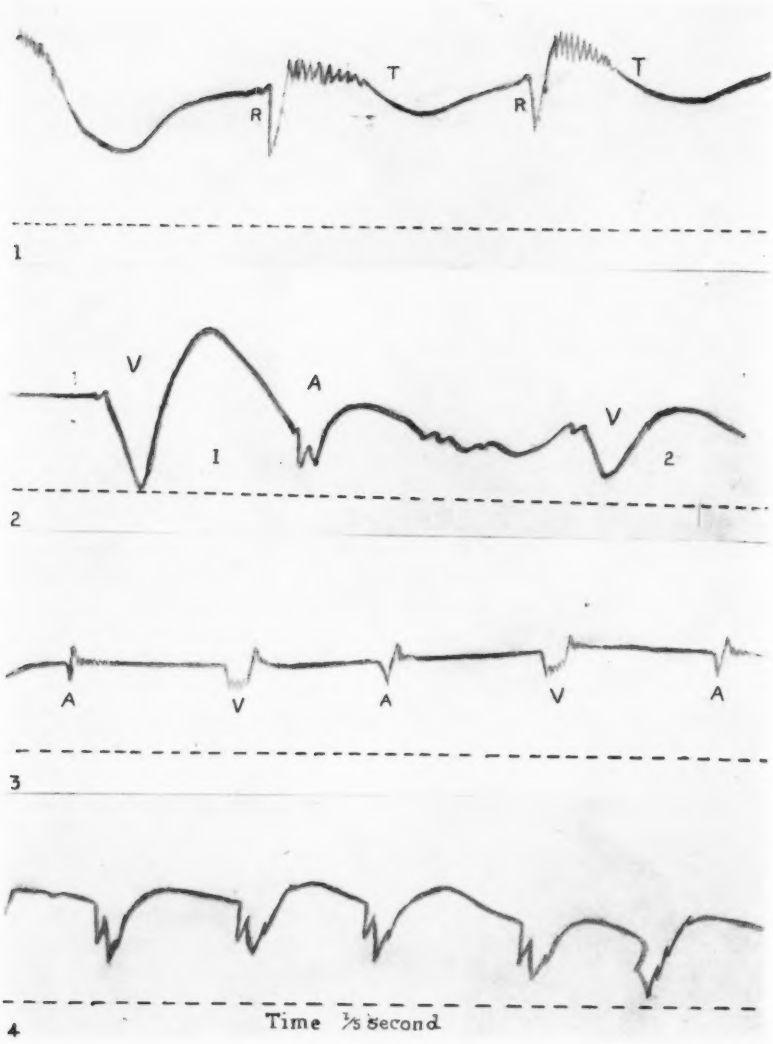

Fig. 1. 1. Ventricular electromyograms showing typical form. The first or *R* complex is made up of rapidly succeeding potential increments. These are fused in the so-called T portion of the record.

2. Electromyogram of the intact whole heart showing *V*, the ventricular response and *A*, the atrial response.

3. Electronuerogram of excised median cardiac nerve of same preparation as in 2. Note two readily distinguishable oscillatory potentials, A, that associated with atrial contraction; B, that associated with ventricular contraction. Note that the rate of potential discharge is now more rapid than in 2. This is usually the case when the effect of the extrinsic nerves is eliminated. The above records are tracings of actual photographs to facilitate reproduction.

4. A series of ventricular electromyograms when the ventricle is being driven by stimulation of the posterior portion of the median cardiac nerve.

active ventricle freed of its median nerve cord is similar to that which has already been described by Carlson for such a preparation when immersed in sea water.

The ventricle of the Limulus heart is surrounded in its posterior two thirds by a membranous covering in which microscopic sections show defi-

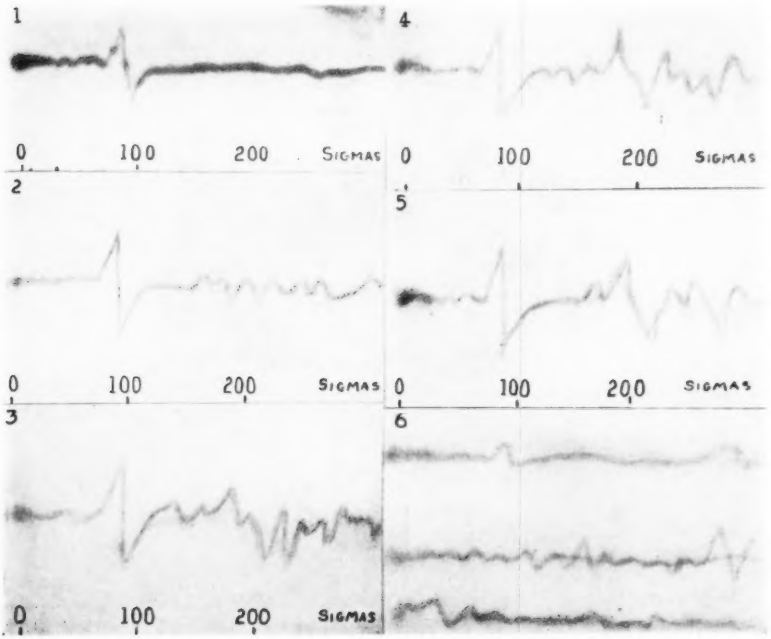

Fig. 2. 1. Potential record from median cardiac nerve electrically stimulated; stimulus strength just below that required to elicit an oscillatory discharge. This potential is referred to in the manuscript as the "first" potential; conduction rate 0.75 m.p.s.

2. Potential record from same nerve. Stronger stimulation results in an increased amplitude of the first potential and also the excitation of an oscillatory potential (second type potential).

3, 4 and 5. Records similar in type to that shown in 2 but with maximal stimulus. Note the constancy of the forms of both the first and the second potential complexes on such stimulation.

6. Record of spontaneous activity in the same nerve.

nite cardiac muscle fibers. (See also Nukada.) Into the space between this covering and the ventricles the venous blood is returned, and from here through definite ostia it enters the ventricle. Examination with the naked eye shows that this membranous structure is definitely contractile

and its contractions can be registered on a kymogram. It is rather difficult to remove the carapace without injury to the thin musculomembranous covering. Electromyograms of the intact heart in good preparations (fig. 1) show a definite potential corresponding in time relationships with the mechanically recorded contraction of the musculomembranous structure. It will be shown later that in good preparations a potential can be recorded from the median cardiac nerve which is an expression of the ac-

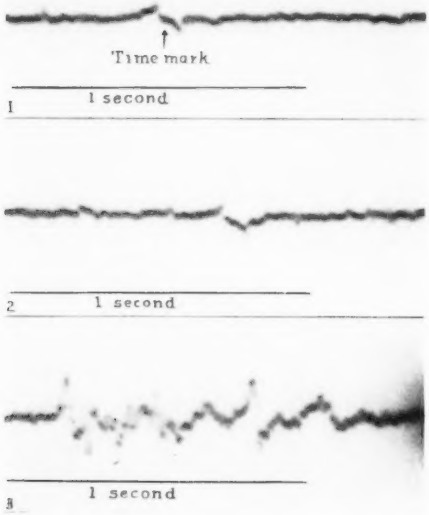

Fig. 3. Continuous record of activity of median cardiac nerve in region of fifth segment. Records 1, 2 and 3 follow each other, being cut here for purposes of reproduction. Time in second, amplification 1200 mm. per mv. In record 1 no nervous activity is apparent, only the time mark showing. In record 2 the low potentials are regarded as those from fibers whose activity results in atrial contraction. In record 3 the ganglion cell activity associated with ventricular contraction is recorded.

tivity of ganglion cells responsible for this muscular activity. Our findings, therefore, confirm the histological and physiological studies of Nukada on this same subject and we agree with him in considering the heart of Limulus to consist essentially of two divisions, an atrium and a ventricle, each of which is contractile.

The potential of the excised median cardiac nerve was recorded for both spontaneous activity and for activity induced by electrical stimula-

tion (figs. 1, 2, and 3). Spontaneous activity reveals itself by a succession of periodic potentials, the total having a duration of approximately 1 to 1.2 second in a heart beating 18 times per minute. With a slow deflection of the base line or on moving film the greatest number of deflections in such a potential is seen to occur near the onset of spontaneous activity, and thereafter they become somewhat less frequent and lower in amplitude (figs. 1 and 2). The oscillatory potential which precedes the first sign of the ventricular electromyogram is always well developed. In good preparations it is also possible to see distinctly a similar potential which begins just before the first sign of atrial electromyogram. Figures

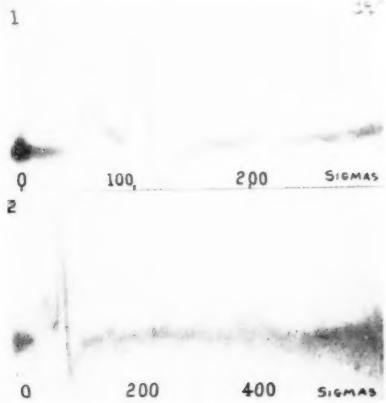

Fig. 4. 1. Record of potential from large fibers, conduction in the postero-aterior direction. Stimulus maximal. First portion of oscillatory potential also evident.

2. Record of potential from large fibers of the same preparation conduction in the antero-posterior direction. Beginning of oscillatory potential also evident.

1 and 2 show the form of the oscillatory potentials associated with contraction, both of the atrium and of the ventricle. The total area of the "ventricular" complex is greater than that of the atrial. The form of the spontaneous oscillatory potential is quite complex and no satisfactory analysis of normal responses seems possible by direct means. The average maximal potential developed is $\frac{1}{10}$ millivolt.

When the potentials of the excised median cardiac nerve are studied by artificial stimulation it is seen that there are at least two potential complexes which together form the autochthonous oscillatory potential. With stimuli just about threshold a potential is elicited which has a conduction rate of 0.75 to 0.5 meter per second at 14°C. This potential will henceforth be referred to as the potential of the first type. The stimulus strength required to elicit it is of the order usually required to stimulate

unmyelinated fibers in vertebrate and invertebrate nerve trunks of similar size, and the form of the potential wave elicited is similar. The potential can be recorded from nearly the entire length of the nerve cord and it conducts anteroposteriorly or posteroanteriorly with equal facility (fig. 4). The absolutely refractory period of the fibers giving rise to this potential is 4 to 6 sigmas and their chronaxie (point electrodes in air) 1 to 2 sigmas. When the stimulating current strength is increased to obtain nearly a maximal potential of the first type an oscillatory potential complex is also to be recorded following each stimulus if these are sufficiently separated in time. This oscillatory complex will be referred to as the second potential. It bears an "all or none" relationship to the first potential; that is, if obtained at all it is maximal. It is of a type and duration similar to the autochthonous oscillatory potential discharge. The complexity of the *spontaneous* oscillatory discharge is such that it is impossible to recognize in it a potential of the first type as a separate entity. The refractory period value for the second potential complex is approximately half its own duration, or 0.5 to 0.6 second; that is, for some time after the nervous impulse of the first type set up by the stimulus directly has excited a potential of the second type, a second nerve impulse of the first type is ineffective even though the nerve fibers concerned have fully recovered. When the median cardiac nerve is stimulated faster than 80 to 100 per minute the ganglion cells responsible for the second potential no longer follow, but take up their own natural rhythm at about the rate of the normal heart beat for the particular temperature. The fibers giving rise to the potentials of the first type follow a much faster rate of stimulation. Stimulation of frequency greater than five per second were not applied, but these fibers followed up to that rate for several minutes.

The first and second potentials can also be differentiated on depression in the presence of pure carbon dioxide. When the median cardiac nerve is placed in this medium the potential of the first type is the first to fail to respond to artificial stimulation. After such failure it is not possible to determine the activity rate of the second potential by electrical stimulation, but it continues to be spontaneously active at its own rhythm for some time. On recovery the second potential is the first to re-appear. This is soon followed by recovery of the first potential after which the latter can be made to "drive" the second potential.

The number of times each ganglion cell is active during a normal oscillatory potential deflection has not been accurately determined. If the interval between what appeared to be corresponding phases in the total complex be taken to represent the interval between successive responses of a ganglion cell, a rough average of 30 times per second is obtained. This is in keeping with the synapse time found for ganglion cells having unmyelinated postganglionic axons (Heinbecker, 1930).

To determine the time relationship between ganglion cell activity and muscular activity in the ventricle the recording electrodes were arranged so that the ground electrode made contact with the ventricular musculature and the grid with a portion of the median cardiac nerve which had been elevated from the posterior region of the ventricle. In this way a record from both nerve and muscle is obtained. A burst of cell activity is seen to begin 30 to 80 sigmas before the commencement of the so-called R complex of the ventricular electromyogram. Inasmuch as this nerve activity ordinarily lasts at least a second it follows that it must persist throughout a considerable portion of the electromyogram. A further demonstration of the relation of the nerve activity to the auricular electromyogram can be obtained in good preparations by using fine silver electrodes, one of which pierces the atrium to rest on the median cardiac nerve, the other of which is attached to the atrium only, the heart being still distended with blood. Ganglion cell activity precedes activity in the atrium by 30 to 80 sigmas as in the ventricle. It is apparent that ganglion cell activity always just precedes activity in the heart muscle and it is certain that the heart beat of Limulus polyphemus is neurogenic in origin. One can also obtain from the excised nerve, spontaneously active, two potential series corresponding to atrial and ventricular responses.

When the heart is artificially driven by direct stimulation of the freed posterior portion of the median cardiac nerve an extra systole is not followed by compensatory pause. This indicates that by such stimulation the pacemaker mechanism of the heart is being excited.

When the form of the spontaneous ventricular electromyogram is recorded by point electrodes both on normal tissue 1 cm. apart, and both within a single segment of the ventricle, it is seen to be composed of a first and second diphasic complex (fig. 1). For the sake of conformity with general nomenclature the first phases of these two diphasic complexes will be referred to as the R and T complexes respectively. The R complex is not simple, but is made up of a rapid succession of potentials corresponding to the rapid potentials elicited from the median nerve cord. Likewise, the T complex is not simple, but made up of a similar but slower group of potentials in good preparations. Because of the slowness of these potentials, relative to the total duration, or because of the fact that they overlap, the T wave tends to be a smooth deflection. While both phases are diphasic the degrees to which the diphasicity is apparent depends on the distance between the recording electrodes and the degree to which contraction is simultaneous in the ventricle at the two recording electrodes. The first, or R complex, appears generally to begin 300 to 600 sigmas before the second or T complex. The beginning of the ventricular myogram corresponds approximately in time relationship with the end of the first phase of the R potential. It is certain that the beginning

of the visible muscular contraction precedes the apparent start of the T potential component.

The positive identification of the actual processes responsible for the form of the electromyogram is beset with some difficulty. There are two possible interpretations of this. The final contracted state may result from successive additions of shortening in the muscle fibers, each fiber responding further in a tetanus with successive impulses from the nerve. Another possible interpretation is that the muscle fibers are not all active from the beginning, but successive groups come into activity as the ganglion cell discharges reach them. The writer favors the first interpretation for the following reasons: 1. There is no appreciable variation in the form of the electromyogram on moving the electrodes to various parts of the contracting ventricle. 2. The ventricle is anatomically a syncytium and it would be contrary to all known biological facts not to have it also act functionally as such. 3. There is no observable variation in the time at which distant parts of the heart become active.

To study the effects of the extrinsic nerves on the activity of the intrinsic ganglion cells the former were exposed near their origin from the brain stem. Shielded electrodes were applied and stimulation effected through an induction coil. In some experiments the vagus and sympathetic nerves were separately stimulated, but in most, both sympathetic and vagus nerves were stimulated together. In such cases a vagus effect was always first obtained, but after some minutes the sympathetic effect over-balanced the vagus effect. Threshold effects were difficult to study, but there was no obvious evidence to indicate that the threshold for the vagus effect was lower than that for the sympathetic effect. Stimulation of the vagus fibers resulted in a lowering of the amplitude of these spontaneously developed potentials as recorded from the median cardiac nerve. There resulted, also, a diminution in their oscillatory rate and in the total duration of their activity. This depression was readily carried to the point of complete extinction of all ganglion cell activities. Purely inotropic effects were possible from submaximal stimuli. Maximal stimulation invariably resulted in chronotropic as well as inotropic depression. After a few minutes of such depression the inhibitory effect generally fails and activity in the ganglion cells manifests itself to a degree even greater than the normal as if during inhibition the ganglion cells accumulate a store of energy greater than their normal complement. Stimulation of the sympathetic fibers altered the spontaneous potential of the median nerve cord by increasing its total amplitude and by increasng the frequency of its oscillatory discharge rate and by lengthening the total duration of the potential (fig. 5). There was also a positive chronotropic effect as evidenced by the more frequent occurrence of the oscillatory discharges. It is important to note that the oscillatory rate on sympathetic stimulation is not the same

as the frequency of the induction coil employed. The changes in the activity of the ganglion cells on vagus and sympathetic stimulation are reflected directly in changes in the activity of the heart muscle. Both inotropic and chronotropic effects in the negative or in the positive direction result, depending on whether the vagus inhibitory or sympathetic augmentatory effects are being produced in the nerve cord. The total duration of the electromyogram is shortened by the vagus effect and increased by the sympathetic effect.

Chronaxie determinations on the normally innervated ventricle by the method described above yielded results varying from 1.5 to 2.5 sigmas.

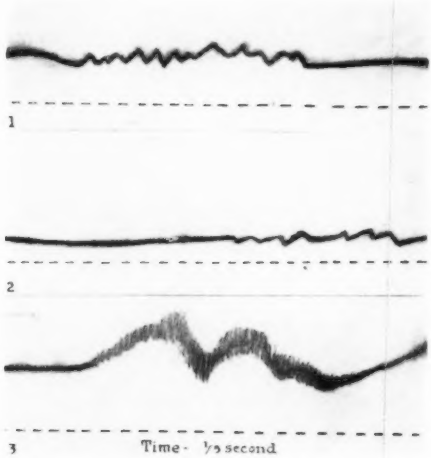

Fig. 5. 1. Record of normal oscillatory potential of the median cardiac nerve which is associated with ventricular contraction. Ventricular rate, 8 per minute.

2. Record of oscillatory potential from same nerve when extrinsic vagus fibers are being submaximally stimulated. Note shortening of potential's duration and some slowing of the oscillatory frequency.

3. Record from the same nerve on stimulation of the extrinsic sympathetic fibers. Note the increased duration of the potential and marked increase in its oscillatory frequency.

Chronaxies determined in the same preparations with the position of the electrode unchanged, but with the ventricle arrested by denervation yielded similar results. Determinations on the same preparations after reëstablishment of muscular activity subsequent to distention of the ventricle, likewise showed no appreciable variation from the normal.

Studies of methylene blue preparations[1] of entire Limulus ventricles

[1] Excellent methylene blue preparations of whole Limulus ventricle with nerves in situ were obtained for these studies from Dr. William Patten. The author is deeply grateful to Doctor Patten for this assistance.

with nerves intact were made to determine the anatomical source of the two potential complexes recorded from the median cardiac nerve. There are essentially two ganglion cell types, one large with axons of large diameter and the other small with axons of correspondingly small diameter. Bishop and Heinbecker (1928) have shown that the threshold of myelinated fibers of the somatic type in vertebrate nerves varies inversely as their diameter. This is considered to depend on conditions purely physical in nature which would apply equally well to unmyelinated fibers. On this basis inference is made that the potential elicited by the least stimulating current is derived from the axons of the large ganglion cells. These axons run nearly the entire length of the median cardiac nerve. This harmonizes well with the fact that the potential of the first type is conducted the entire length of the nerve cord and in both directions with equal facility. The second potential group composed of potentials of a repetitive type are inferred to be derived from the small axons of the small ganglion cells. There is no recognizable difference in cell types to separately account for the potentials associated with atrial as contrasted with ventricular muscular activity.

The anatomical distribution of the ganglion cells in the median nerve cord when considered in relation to the functional findings permits inferences as to the function of these cells. The large ganglion cells are most numerous in the region of the 4th, 5th and 6th ventricular segments, the region of the heart normally the seat of greatest automaticity or the presumptive pacemaker of the heart. It is therefore reasonable to consider that the large ganglion cells are the pacemaker cells. The association of the nerve cord with ventricular muscle contraction is of wider extent, occurring practically throughout the length of the nerve cord. It is least marked in the first two segments. In this region of the ventricle the association of oscillatory nerve impulses in the lateral nerve trunks as well as the median nerve cord has been established by direct observation. It is significant that small ganglion cells are distributed throughout the length of the median nerve cord, but with their number least in the region of the 1st and 2nd segments. Again it seems reasonable to consider the activity of the small ganglion cells which is repetitive in type to be directly responsible for the impulses causing muscular contraction. The oscillatory potentials are produced when all connections with extrinsic nerves are removed. In other words, their activity is autochthonous. It has been shown that the groups of oscillatory potentials can follow the stimuli applied to the large axons of the large ganglion cells up to a rate several times their normal frequency. It is not possible to directly and separately demonstrate the autochthonous rhythm of the large ganglion cells. The indirect evidence of their activity, as shown through their pacemaker properties, is overwhelmingly in favor of the inference that they too are automatically active.

DISCUSSION. The experimental finding that the deganglionated ventricle of Limulus can be made rhythmically active by distention is in agreement with that of Dubisson (1931). The activity under such circumstances is peristaltic in type and thus rather similar to that activity in the embryonic Limulus heart before ganglion cells have entered it, as described by Carlson and Meek (1908). Only after ganglion cells enter the heart is its activity modified to the form found in the normal adult. This is similar to the course of events in the vertebrate heart and one must, therefore, accept with reservations the argument that the vertebrate heart is myogenic in the adult because its rhythmic activity commences before the entrance of nerve elements. Limulus heart retains in its adult musculature the potentialities of autochthonous rhythm. This is controlled and modified by the intrinsic and extrinsic heart nerves. Even the vertebrate heart (turtle ventricle where there are no ganglion cells) becomes rhythmic with but slight change in salt balance.

Our studies on the intrinsic ganglion cells of the median nerve cord and the effect of the extrinsic nerves on their activity permit inferences as to the nature of the interaction of the two types of ganglion cells and also as to the locus of action of the extrinsic nerve fibers on the intrinsic nerve mechanism. It has been demonstrated in those experiments in which the nerve was directly stimulated, that activity in the large nerve fibers and consequently in the large ganglion cells can initiate activity in the small nerve fibers arising from the small ganglion cells. For reasons already presented this is interpreted to indicate that the large ganglion cells acting as pacemaker cells regulate the rhythm of the small ganglion cells which directly innervate the heart. Experimentally, changes in the activity rate of the small ganglion cells by the large can only be obtained in a positive direction; that is, as an increase from the basic rhythm of the small ganglion cells. No experimental evidence that activity in the large fibers can result in a depression of the activity of the small ganglion cells has ever been encountered. The limit to which the large ganglion cells drive the small ganglion cells is fairly definite, in our experiment to not more than 80 to 100 times per minute. Above this rate the small ganglion cells fall back to their own intrinsic rhythm which approximates that of the normal heart. The reason for failure of influence of the large ganglion cells at higher rates of stimulation lies in the fact that the small ganglion cells as a group are refractory for approximately half the duration of their oscillatory discharge period. At an average heart rate this half period is 500 to 600 sigmas which is also the interval between stimuli at 80 to 100 per minute, the rate at which the small cells fail to follow the large ones.

When the extrinsic nerves to the heart are cut it generally beats more rapidly and the atrio-ventricular interval is shortened. This indicates that normally the extrinsic fibers slow the heart and increase the interval

between contraction of the atrium and of the ventricle. The chronotropic influences are naturally inferred to act directly on the large ganglion or pacemaker cells. But inasmuch as the small ganglion cells have been shown to have an autochthonous rhythm independently of the large ganglion cells it follows that in order that the heart be *stopped* by extrinsic fibers these must also act directly on the small ganglion cells. The effect on the latter is first an inotropic one, but with stronger stimulation it also becomes chronotropic. Very definite inotropic depression without chronotropic modifications can be produced by stimulation of the elevated median cardiac nerve in the region of the first three ventricular segments. In this our own experimental results fully corroborate those of Hoffmann (1911). The duration of the oscillatory discharge of the median cardiac nerve under such circumstances is lessened as is the duration of the ventricular electromyograms. Our findings in these experiments are regarded as further evidence in favor of the interpretation that the oscillations in the electromyogram record indicate additions of shortenings in contracting fibers in a true tetanus rather than the addition of successively contracting fiber groups. Under such inhibition the electromyograms show no appreciable alterations other than shortening of total duration.

Stimulation of the intrinsic nerve with different strengths and numbers of stimuli indicates that here, as in the vagus effect on the vertebrate heart, the number of fiber-stimuli determines the ultimate effect; that is, increase of number of fibers stimulated or of number of times each fiber is stimulated has the same result. Maximal single stimuli can have more than a threshold effect. It is therefore considered that more than one extrinsic fiber must act on each ganglion cell. The effect of the sympathetic fibers is quantitatively similar to the effect of the vagus fibers, but qualitatively opposite. The above scheme (fig. 6) presents a simple, but possible, explanation of the activity of the intrinsic ganglion cells upon each other and of the extrinsic fibers upon them. Future investigation may add further complexities; the present outline affords at least a working hypothesis.

It is of interest to consider the locus of action of the extrinsic fibers on the intrinsic ganglion cells. The fact that on stimulation of the extrinsic vagus fibers all activity in the median cord can be abolished indicates that the extrinsic nerve impulses do not act on synapses, but on the ganglion cells directly. If not, intrinsic rhythmic impulses of these cells could still be propagated along their axons. It is also significant that in one sense vagus depression does not inhibit the excitatory process of the ganglion cells. These cells tend to break from their inhibited state and on release show an *increased* activity. The type of inhibition found to exist in the Limulus heart is apparently different from that which is postulated by Sherrington and others for motor cells in the spinal cord. By these authors the central excitatory state and the central inhibitory state are

considered to exist at the synapse. It is highly probable that in Limulus the effects of the sympathetic extrinsic fibers are accomplished at the same locus as the vagus inhibitory effects.

The chronaxie of the contracting deganglionated ventricle has been found by us to be similar to that of the normally innervated ventricle. The method employed is the one most free of experimental difficulties and other sources of error. The results are at variance with those reported by Dubisson (1931) who found two chronaxies for these two experimental conditions. He obtained a chronaxie 8 to 25 times greater for the arrested deganglionated ventricle than for the normal. One is tempted to refer to the two chronaxies for the muscle reported by Rushton (1930). Lapicque

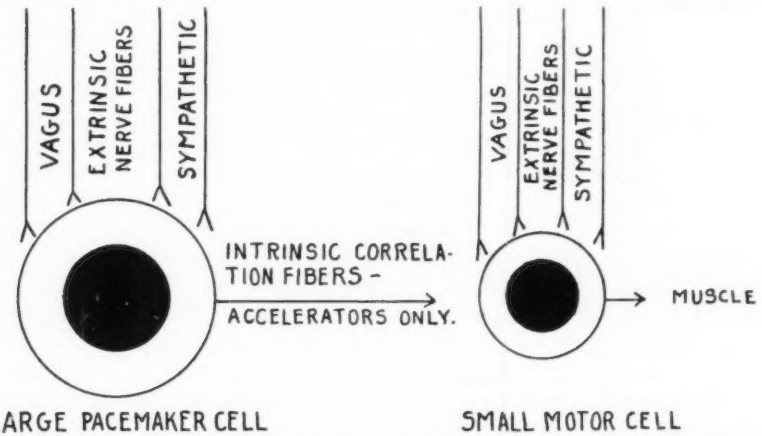

Fig. 6. Diagrammatic representation of possible connections of extrinsic nerves with the intrinsic nerve mechanism of the median cardiac nerve concerned with ventricular contraction. Ganglion cells responsible for atrial contraction are, by inference, considered to be similarly connected. Presumably, also, correlation fibers would be necessary to regulate atrio-ventricular relationships.

(1931) has recently claimed that the two muscle chronaxies are dependent upon the type of circuit employed. It seems possible that there may have been sufficient difference in Dubisson's and our own experimental conditions to account for the differences in our result. At any rate, it is not possible in the face of our experimental results to accept Dubisson's interpretation of the action of the extrinsic ganglia of Limulus on the heart muscle. He considers that their function is to control the "quickness of excitability" of the musculature and this in turn determines its automaticity.

Dubisson and Monnier (1931) consider that the normal electrocardiogram of Limulus polyphemus does not indicate an oscillatory type of con-

traction. In view of the demonstrated oscillatory nature of the discharge from the ganglion cells and also the form of the electrocardiogram obtained by us it is impossible to agree with their interpretation. Our interpretation is in agreement with that of Hoffmann (1911) and in part with that of Garrey (1932).

From the standpoint of comparative physiology two phases of the findings of this study on Limulus may be further considered. The first concerns itself with the general significance of the findings that the extrinsic fibers of the heart, both vagus and sympathetic, do not possess the ordinary relationship of preganglionic fibers to the cells of the median cardiac nerve. In the vertebrate heart it has been customary to consider that the cervical vagus fibers are preganglionic to the cells within the heart, and in the sympathetic ganglia of vertebrates the experimental evidence indicates that activity in preganglionic fibers is transferred through the synapse to postganglionic fibers without any change in rhythm (Heinbecker, 1930). It might be inferred that in the vertebrate heart, impulses from the vagus propagate across synapses to heart ganglion cells in a similar one-to-one relationship. The perfect correspondence in function between the invertebrate and the vertebrate extrinsic heart fibers makes it highly probable that the anatomical relationship of the structures concerned are identical. It is not to be excluded that even in the vertebrate heart, the effect of the vagus impulses is to slow the intrinsic rhythm of heart ganglion cells, but so far it has not been possible to make direct observations in the vertebrate heart. Because the ganglia of the lungs and intestines of the vertebrate are similar in their ontogeny to the ganglia of the heart the suggestion is made that in these also the same relationship between extrinsic and intrinsic nerve elements may exist.

The second point we wish to emphasize has to do with the differentiation between the inotropic and chronotropic mechanisms. In a previous communication (Heinbecker, 1931) it was demonstrated that a recognizable component of the conducted action potential of the turtle cervical vagus trunk, probably derived from myelinated fibers, was associated with a purely inotropic effect on the atrium. A different potential component derived from unmyelinated fibers was associated with chronotropic effects. The evidence indicated that activity in the unmyelinated fibers also produced a further inotropic effect. While it is recognized that results of experiments on the invertebrate heart are not necessarily directly transferable to the invertebrate heart, the perfect similarity of functional effects of the extrinsic fibers in the two instances again makes a similarity of anatomical basis highly probable The results in the case of Limulus definitely indicate that the extrinsic nerve fibers act on at least two different sets of ganglion cells. In the vertebrate heart also a similar condition seems to obtain. We thus are inclined to support the hypothesis of Tiger-

stedt (1921) and others whereby it is considered that fibers responsible for purely inotropic effects are not the same as those responsible for negative chronotropic effects and that they act on different structures in the vertebrate heart. Insofar as the small ganglion cells are concerned, our findings would also support the hypothesis of Hering (1905) and Henderson (1906) who consider that the different effects, inotropic and chronotropic, of the extrinsic fibers are expressions of one and the same kind of influence, the degree rather than the type of influence being the determining factor.

SUMMARY

The ventricle of Limulus polyphemus freed of ganglion cells contracts rhythmically if normally distended.

The contraction of the normally innervated ventricle is practically simultaneous throughout its length.

The contraction wave of the deganglionated ventricle propagates as a slow peristalsis.

The adult Limulus heart is neurogenic.

The Limulus heart consists essentially of an atrium and a ventricle, each of which is contractile.

An analysis of the potential of the median cardiac nerve is presented. It consists essentially of two components, one derived from the axons of large pacemaker ganglion cells, the other from the axons of small motor ganglion cells.

The spontaneous activity of the ganglion cells of the median nerve cord is oscillatory in type.

An analysis of the ventricular electromyogram shows the contraction process to be a tetanus.

Records have been made of the effect of the extrinsic nerves on the potential of the median cardiac nerve.

A scheme of the manner of action of the intrinsic ganglion cells of the median nerve cord upon each other and of the extrinsic nerves on the intrinsic nerve mechanism is presented.

The findings of this research are discussed from the standpoint of comparative heart physiology.

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ON THE HUMORAL AGENTS CONCERNED IN THE CAUSATION OF GALL-BLADDER CONTRACTION

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When one considers the humoral agents concerned in the excitation of the digestive glands, one thinks of specific hormones and of secretagogues, the latter being present in the food or arising during the digestion of the food and acting after being absorbed into the blood or lymph. Although we believe that a hormone mechanism for gall-bladder contraction and evacuation has been established (Ivy and Oldberg, 1928; Lueth, Ivy and Kloster, 1929; Ivy, Drewyer and Orndoff, 1930), the possibility that substances secretagogue-like in nature may be concerned must be investigated. That this possibility must be considered is shown by the observation of Whitaker (1929) to the effect that emulsified olive oil injected intravenously causes some evacuation of the gall bladder. However, Whitaker questioned the physiologic nature of the response because emulsified liquid petrolatum produced the same effect intravenously, but is inactive by mouth. Further, Higgins and Wilhelmj (1929) obtained no evacuation on injecting various emulsified fats intravenously, and Silverman and Dennis (1928) observed evacuation in man without a significant change in blood fat. Boyden (1926) has observed evacuation after a fat meal in cats with the thoracic duct tied near the cisterna.

Since it has been shown that sudden changes in blood pressure which influence liver volume also exert an influence upon intra-gall-bladder pressure, it was thought that the discrepancy between the findings of Whitaker and Higgins and Wilhelmj might be due to the size of the fat droplets in the injected emulsion or to the vascular reactions induced by the injection.

METHODS. We have studied the effect of injections of egg yolk, emulsified olive oil, fatty chyle, and various digests upon blood pressure, liver volume change, and intra-gall-bladder pressure. Dogs were anesthetized with barbital and the carotid artery and gall bladder were prepared to record changes in pressure. Records of changes in liver volume were made by placing a small rubber balloon between the lobes of the liver after the

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method of Bainbridge and Dale (1905). The response to cholecystokinin was obtained with the usual result of no appreciable change in blood pressure, no change in liver volume, and a marked rise in intra-gall-bladder pressure, and then the other substances to be tested were injected.

From 1 to 4 cc. of a 50 per cent olive oil emulsion in 5 per cent acacia and 0.2 per cent sodium benzoate were injected. From 2 to 4 cc. of egg yolk freed from egg white by washing, either undiluted or diluted one-half with 0.9 per cent sodium chloride solution were injected. From 10 to 120 cc. of fatty chyle (1.0–1.5 per cent) collected from five to seven hours after a meal of egg yolk and cream (six egg yolks and 200 cc. of cream) were also injected. We also injected from 3 to 5 cc. of 0.2 per cent hydrochloric acid solution and 10 cc. of 5 per cent sodium chloride solution intravenously, since dilute hydrochloric acid was used in the cross-circulation experiments of Ivy and Oldberg (1928) and it is reported that hypertonic sodium chloride solution intravenously (Orr, Johnstone and Haden, 1931; Reid and Ivy, 1931) excites contractions of the small intestine. The injections were given during a period of about one minute to dogs weighing from 10 to 20 kilos.

On the assumption that secretagogue-like substances occurring in *in vitro* digests of foods known to activate the gall bladder *in vivo* would resemble cholecystokinin in their chemical properties, a process of extraction was applied to these digests which very closely resembles the method used in extracting cholecystokinin from duodenal mucosa. The digests and extracts were prepared as follows: Three hundred cubic centimeters of "top milk" were mixed with the yolks of three eggs, 2 grams sodium carbonate, and 2 grams "pancreatin." The whole was digested twenty-four hours at 37°C. The digest was treated with an excess of sodium chloride, filtered, and the precipitate extracted with 800 cc. of 95 per cent ethyl alcohol. The alcohol-soluble elements were filtered off, diluted with 100 cc. of water, and then vacuum distilled at 60°C. until all of the alcohol had been removed. Portions of the raw digest and also of the solution prepared above were injected intravenously.

A similar extract of 4 grams of "pancreatin" was made in order to rule out the introduction of any preformed secretagogue-like substance existing in "pancreatin." Amounts equivalent to 2 grams were injected intravenously into each of two dogs.

A digest of olive oil consisting of 50 cc. of olive oil emulsified with 2 per cent acacia, 500 cc. of water, 2 grams of "pancreatin," and 0.5 gram sodium carbonate was incubated forty-eight hours. Sufficient toluol was added to prevent bacterial action. This was removed later by bubbling air through the mixture. Very small amounts were injected intravenously since the digest was quite toxic.

Similar digests were prepared using olive oil without acacia, but with

"pancreatin" and sodium carbonate buffer in the same proportions. Small amounts of these were also injected intravenously.

Digests using fresh pancreatic juice, olive oil, and sodium carbonate solutions were also prepared. The proportion of oil to juice was five to one and the carbonate buffer was added to double the volume. These were also digested from twenty-four to forty-eight hours and small amounts administered intravenously. Forty cubic centimeter quantities were also injected into the lumen of the lower ileum and after twenty minutes, into the lumen of the duodenum also.

An extract of one of these digests which was known to cause an outflow of dark bile when introduced into the human duodenum in two instances, was also prepared. In preparing this, the cholecystokinin technique as described above was also used. Amounts of the extract equivalent to twenty times the dose of the original digest capable of causing the appearance of dark bile in the duodenum of the human were injected intravenously into two dogs.

Oleic acid (C.P.) was injected intravenously in amounts of from 1 to 5 cc.

Glycerol in doses varying from 3 to 5 cc. was administered intravenously also.

RESULTS. *Egg yolk.* Egg yolk intravenously in four dogs caused no change in blood pressure, liver volume, or intra-gall-bladder pressure. In the fifth dog, however, a marked fall in blood pressure with a simultaneous increase in liver volume and intra-gall-bladder pressure occurred which was like the changes noted following the injections of emulsified olive oil (see fig. 1).

Emulsified olive oil. In nine dogs the intravenous injection of olive oil, after an appreciable latent period, caused a fall in blood pressure, but a rise in liver volume and intra-gall-bladder pressure, the three changes being closely correlated in regard to degree and duration (fig. 1). The amount of change varied from dog to dog and with the dose. Hyperpnea was evident and the intestines and liver became cyanotic when the larger doses were given. Fat embolism was evidently produced. A second injection of the emulsion in the same amount rarely produced any change; but a larger amount produced changes similar to those which occurred at the first injection. The non-saponifiable fraction of olive oil produced no changes. When the olive oil emulsion was injected into the femoral artery, no response was obtained until the amount was eight times that necessary to produce a response when given into the femoral vein. In a dog that was quite "refractory" to cholecystokinin (five or six average dog doses of cholecystokinin being required to produce a gall-bladder response), the typical response was obtained with 1 cc. of the olive oil emulsion. The intravenous injection of air produced changes identical with those produced by the emulsion, with the exception that the amount of olive oil required

to produce the changes was not fatal whereas the amount of air required was usually fatal. *In one dog with marked fatty degeneration and cirrhosis of the liver, no change in liver volume was recorded after the injection of the emulsion.* The gall bladder showed a loss of tone or a decrease in intra-gall-bladder pressure. The gall bladder of this dog responded to cholecystokinin. A loss oftone of the gall bladder was usually, but not always, observed after the injection of the emulsion as the blood pressure slowly returned to normal. In some tracings this apparent loss of tone was associated with a decrease in liver volume, but not always.

Dilute hydrochloric acid and hypertonic sodium chloride solution. The intravenous injection of dilute hydrochloric acid sufficient to cause some hyperpnea failed to cause a change in intra-gall-bladder pressure.

The intravenous injection of 10 cc. of 5 per cent sodium chloride solution was also without effect.

Fresh pancreatic juice (10 to 50 cc.) intravenously has no effect on the gall bladder. Bile or bile salts decrease the tone of the gall bladder.

Chyle. The intravenous injection of from 10 to 120 cc. of chyle containing from 1 to 2.5 per cent fat caused no change in intra-gall-bladder pressure in eight experiments (fig. 1).

In order to avoid the possibility that anesthesia might depress the gall-bladder musculature and mask a slight motor reaction that chyle might produce, we performed two experiments on unanesthetized dogs. The gall bladder was visualized with tetraiodophenolphthalein and then 45 cc. of 2.5 per cent fatty chyle were injected intravenously. The gall-bladder shadow did not decrease in size within a half-hour. Measurements by a planimeter showed that the shadow increased by about five and ten per cent. Then cholecystokinin was injected intravenously, three doses at ten minute intervals, and within a half-hour the gall-bladder shadow was reduced eighty-two per cent in one dog and thirty-five per cent in the other.

Fig. 1. Each of the three tracings in this figure shows a record of liver volume changes, *L.V.*, blood pressure, *B.P.*, and intra-gall-bladder pressure, *G.B.* In the upper tracing, 75 cc. of chyle (1.5 per cent fat) were injected during the presence of marked tonus changes (3.2 cm. of bile pressure) in the gall bladder. It is evident that no contraction resulted and the rate and amplitude of the tonus rhythm was not definitely altered. On the injection of cholecystokinin (0.8 mgm. in toto) a contraction of the gall bladder resulted with the usual disappearance of the tonus rhythm during the contraction which reappeared during the relaxation phase. In the middle tracing cholecystokinin was injected which was followed by egg yolk. This was the only time that the egg yolk appeared to be toxic. Note the rise in liver volume with the fall in blood pressure and the rise in intra-gall-bladder pressure which is mechanically induced by the increase in liver volume. The lower tracing shows that egg yolk may be injected intravenously without inducing any change and that a "coarse" olive oil emulsion causes a change in intra-gall-bladder pressure which corresponds closely with the increase in liver volume and fall in blood pressure due to pulmonary embolism.

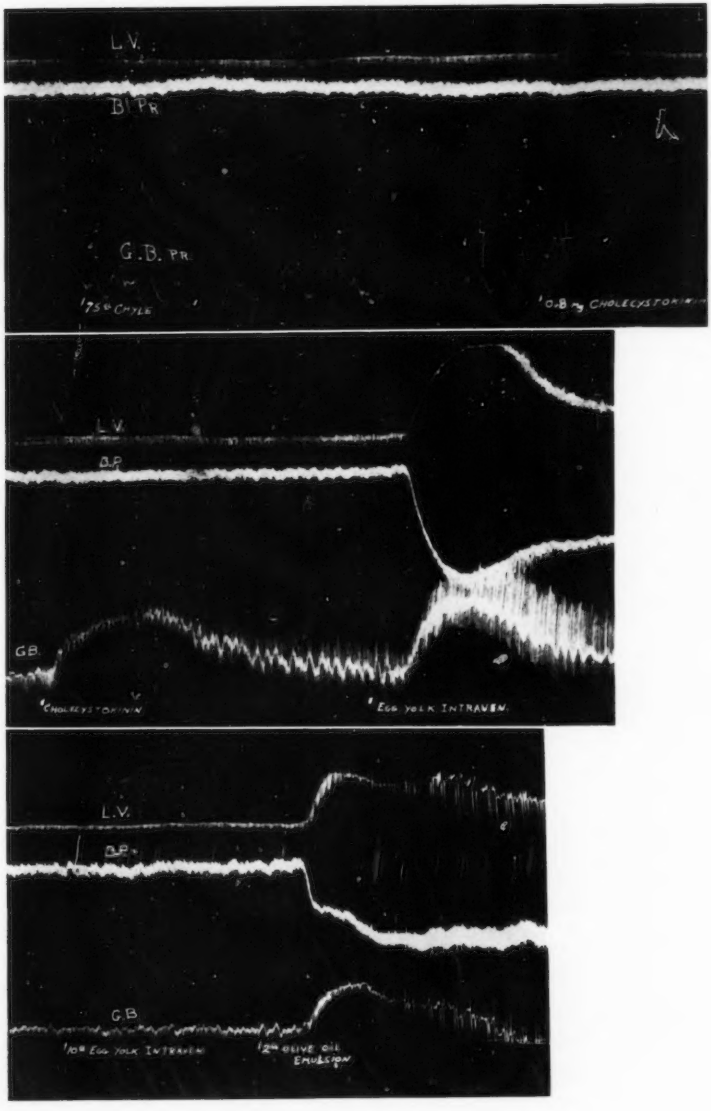
L.V.
B.P.
G.B. P.R.
1/75th CYCLE
1 c.c. of CHOLECYSTOKININ
L.V.
B.P.
G.B.
CHOLECYSTOKININ
EGG YOLK INTRAVEN.
L.V.
B.P.
G.B.
1/10th EGG YOLK INTRAVEN.
1/2nd OLIVE OIL EMULSION

Fig. 1

These experiments show that fatty chyle does not contain a principle which causes gall-bladder contraction (fig. 2). It should be stated that in one experiment the chyle was collected from an unanesthetized "chronic" thoracic duct fistula dog, which was done to control the possibility that anesthesia might possibly depress the formation of the hormone.

"Pancreatin" digest of "top milk" and egg yolk. The injection of 10 cc. of the raw digest produced the lung embolism effect described elsewhere in this paper with parallel rises in intra-gall-bladder pressure and liver volume and with dyspnea and a fall in blood pressure. Intravenous injections of the extracts of this digest were made in increasing amounts up to those equivalent to one-third of the total extract. In two dogs no ef-

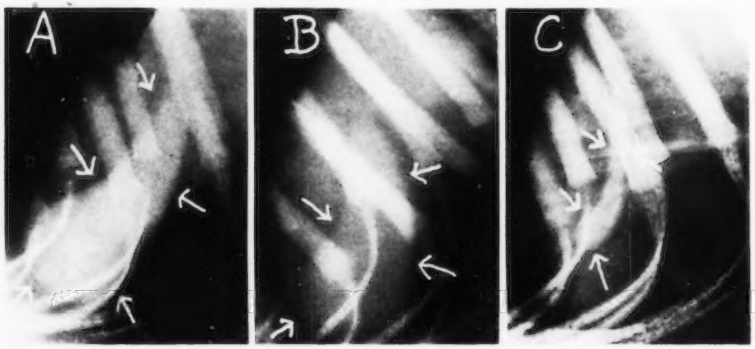

Fig. 2. These are roentgenograms of the gall bladder visualized with tetraiodophenolphthalein showing that the intravenous injection of 45 cc. of 2.5 per cent fatty chyle, obtained after feeding egg yolks and cream, into unanesthetized dogs does not cause contraction and evacuation of the gall bladder. A, control (8:00 a.m.) picture; B, half-hour (8:30 a.m). after the intravenous injection of 45 cc. of chyle (8:30 a.m.); C, half-hour (9:00 a.m.) after the injection of three doses of cholecystokinin at ten-minute intervals (9:00, 9:10, and 9:20).

fects were noted. In two other dogs a very slight increase in intra-gall-bladder tension, without any apparent change in liver volume occurred upon the first injection, but this could not be repeated and was so slight as to be of very doubtful physiologic significance.

A definite pressor effect was noted in all four dogs coincident with these injections. This was shown later to be due to the lecithin obtained from the egg yolk and not removed by the extraction process. A prepared solution of lecithin produced exactly similar results.

Extract of "pancreatin." The injection of extract equivalent to 2 grams of "pancreatin" prepared as above was negative in two dogs in all respects.

"Pancreatin" digest of olive oil acacia emulsion. The intravenous in-

jection of 2 cc. of the watery layer of the raw digest caused a definite contraction of the gall bladder with relatively little change in the liver volume (three dogs). However, the blood pressure fell almost to zero and returned slowly so that the result *per se* is of little significance.

"Pancreatin" digest of non-emulsified olive oil. Intravenous injection of 2 cc. of the watery layer of the raw digest in two dogs caused a very pronounced fall in the blood pressure without any noticeable alteration in intra-gall-bladder tension or liver volume.

"Pancreatic juice digest" of non-emulsified olive oil. The intravenous injection of 0.5 to 5 cc. of the raw digest caused a marked lowering of the blood pressure in two dogs. There was no change in the intra-gall-bladder tension or in the liver volume in either case.

The injection of 40 cc. of raw digest into the lower ileum, with a period of fifteen to twenty minutes allowed for absorption, was followed by no change in either intra-gall-bladder pressure, liver volume, or blood pressure in any of the five dogs so treated.

The intraduodenal injections of 40 cc. of raw digest followed by a similar absorptive period were also negative in four cases. In one case there was a slight increase in the tonus waves of the gall bladder as observed by Ivy and Oldberg (1928).

Extract of pancreatic juice olive oil digest. The intravenous administration of this extract was followed by entirely negative results as to change in intra-gall-bladder pressure, liver volume and blood pressure in two dogs.

Oleic acid. Upon intravenous injection in two dogs, oleic acid was found to depress the blood pressure moderately but not to affect the intra-gall-bladder pressure or the liver volume.

Glycerol. Intravenous injections of glycerol in doses of from 3 to 5 cc. were found to be ineffective in altering either intra-gall-bladder pressure, liver volume, or blood pressure to any appreciable degree.

Discussion. The droplets in the egg yolk emulsion are invisible, and do not measure more than 2 microns in diameter. Many of the droplets in the olive oil emulsion are visible. We believe this physical difference is primarily responsible for the difference in the results obtained with the two emulsions, olive oil emulsion causing fat embolism and the egg yolk not. In the one dog which reacted to egg yolk as though olive oil emulsion had been injected, we can only suggest that the dog was sensitive to the protein of egg yolk, since the egg yolk emulsion was not "cracked" but "normal" physically.

These observations, we believe, show that the rise in intra-gall-bladder pressure which results after the intravenous injection of olive oil emulsion is due to a change in liver volume and hence is an artifact as was suggested by Whitaker. The failure of Higgins and Wilhelmj to observe evacuation

of the gall bladder after injecting fat emulsions intravenously was probably due to the possibility that their fat emulsions were composed of very small droplets.

From the results obtained with extracts of various "motor meals" for gall-bladder evacuation, no active secretagogue-like substance was demonstrated to be present in them by our methods of extraction. In the case of the "top milk" and egg digest, a slight but unrepeatable contraction occurred in two dogs. We believe that these were artifacts. The methods of digestion involve the use of lipase solutions containing definite amounts of vaso-dilator substance which along with the presence of toxic substances in the end-products of digestion, render the resulting digests unsuitable for intravenous administration. The extraction of the digests so as to remove these vaso-dilating substances presupposes that the gall-bladder contracting substance, which may or may not be present, will not be lost or destroyed by the process of extraction. This is perhaps too great an assumption. However, the fact that the products of the digestion of olive oil by a lipase were toxic, glycerol and oleic acid being inactive when injected intravenously, seemed to justify the extraction of the digest by a process which was known to remove a non-toxic gall-bladder excitant from a protein-lipin containing matrix, namely, duodenal mucosa. It was also thought that either some impurity common to olive oil or some protein substance of the pancreatic juice might be the source for the secretagogue-like substance. Such extracts did not cause the gall bladder to contract on intravenous administration.

The fact that egg yolk when taken by mouth is a potent gall-bladder evacuant, and intravenously has no specific effect upon the gall-bladder musculature, shows that there is no substance preformed in the egg yolk which causes the gall bladder to contract. It should be mentioned that one of us (A.C.I.) with Doctor Silverman of New Orleans has extracted the yolks of twelve eggs by the method employed for the preparation of cholecystokinin and failed to obtain a product which when given intravenously caused the gall bladder to contract. The fact that chyle, collected after feeding egg yolk and cream, and then injected intravenously with no effect on the gall bladder, shows that the products of the digestion of egg yolk and cream which are absorbed into the lymph are not effective in causing gall-bladder contraction in anesthetized dogs. The experimental worth of this statement is enhanced by keeping in mind that the 45 and 120 cc. volumes of fatty chyle, which were injected within a period of three minutes represent the total chyle flow from the thoracic duct for a period of two hours or longer, i.e., the factor of dilution or of the possibility of a subminimal dose of the humoral agent in the chyle injected cannot be considered to be a reason for the failure of the gall bladder to contract. That anesthesia is not a factor responsible for the negative results obtained with

chyle is shown conclusively by the experiments on the unanesthetized dogs in which the gall bladder was visualized by tetraiodophenolphthalein. The fact that none of the extracts of *in vitro* digests of various "gall-bladder motor meals" were effective in causing gall-bladder contraction suggests but does not prove that pancreatic digestion of a motor meal does not produce an intermediate product which upon absorption into the blood stream causes the gall bladder to contract. It must be recognized that it is still possible for some as yet unrecognized product of digestion of egg yolk or olive oil to be absorbed into the portal blood which may cause the gall bladder to contract. Before this can be determined, it will be necessary for us to first learn how to identify cholecystokinin chemically and to show that another gall-bladder exciting substance or group of substances, is present in the portal blood. However, these results strongly indicate that a hormone is the sole humoral agent concerned in the causation of gall-bladder contraction and evacuation after the ingestion of egg yolk, cream and olive oil.

SUMMARY AND CONCLUSIONS

An attempt has been made to determine whether some product of the digestion of fat and egg yolk may excite the gall bladder to contract on being absorbed. This was done to ascertain whether some humoral agent other than cholecystokinin may be concerned in gall-bladder evacuation on ingesting egg yolk and cream or olive oil. Our results indicate that no other humoral agent than cholecystokinin is concerned in gall-bladder contraction and evacuation.

1. Egg yolk injected intravenously usually has no effect on liver volume, intra-gall-bladder pressure and blood pressure.

2. Emulsified olive oil injected intravenously in doses which increase the liver volume causes an artificial or mechanical rise in intra-gall-bladder pressure.

3. From 3 to 5 cc. of 0.2 per cent hydrochloric acid solution and 10 cc. of a 5 per cent sodium chloride solution as well as fresh pancreatic juice (10 to 50 cc.) have no effect on the gall bladder when injected intravenously. Bile or bile salts injected intravenously tend to decrease the tone of the gall bladder.

4. Olive oil and egg yolk digests were too toxic to use intravenously for this work. When such digests are extracted by the method for obtaining cholecystokinin from the duodenal mucosa, no gall-bladder excitant is obtained.

5. Oleic acid (1 to 5 cc.) and glycerol (3 to 5 cc.) given intravenously have no effect on the gall bladder or liver volume.

6. Chyle containing from 1.0 to 2.5 per cent fat, and obtained from a dog after being fed egg yolks and cream, on being injected intravenously in

amounts of from 10 to 120 cc. did not cause the gall bladder to contract and evacuate in anesthetised or unanesthetised dogs.

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THE CENTRAL INHIBITORY MECHANISM INVESTIGATED BY MEANS OF ANTIDROMIC IMPULSES

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The reciprocal innervation of antagonistic muscle groups in the spinal reflexes has been the subject of many researches since the classical experiments of Sherrington (1906). From these investigations it has become a familiar fact that when the flexion reflex is evoked in the hind limb, not only is there a discharge of motor impulses from the flexor center, but an inhibition of activity in the extensor center. Conversely the crossed extension reflex includes, besides excitation of extensors, the inhibition of flexors. To explain this phenomenon Graham Brown (1914) suggested, as the essential mechanism, the side-fibrils of Golgi, which von Lenhossék (1895) described as given off from the axons of mammalian spinal motor neurones near their point of passing from the gray into the white matter of the cord. These might serve to conduct impulses into the antagonistic centers, where they could exert a central inhibitory effect. Thus excitation of a central motor neurone would not only send impulses down the axon to the muscle, but would simultaneously send impulses over the side-fibrils to the synapses in the antagonistic center where inhibition of the opposing muscle group would be effected. This suggestion was utilized in a schema developed by Forbes (1921, 1922) in an attempt to test the question whether a conceivable mechanism could explain the known facts of spinal reflexes in terms of the established properties of conduction in peripheral nerve and muscle.

Dr. Philip Bard recently suggested that the question whether the side-fibrils of Golgi possessed the property of inhibiting the antagonistic center might be tested directly by the method of "back-firing" antidromic impulses up the motor neurones, devised by Denny-Brown (1928, 1929). This method in the hands of Eccles and Sherrington (1931) has proved extraordinarily fruitful, and their experiments have clearly revealed that impulses thus sent back into the motor neurone reach the center and exert a marked effect therein. According to Doctor Bard's suggestion it should be a simple matter to send antidromic impulses into the flexor motor center while the crossed extension reflex was being evoked. Since the side-fibrils

are given off from the axon, there is every reason to expect that the antidromic impulse will be conducted over them to their terminations. If it is a function of those side-fibrils which arise from the flexor motor neurones to inhibit the extensor center, this procedure should result in inhibition of the crossed extension reflex. To deliver antidromic impulses into the flexor center without complicating the effect by afferent impulses, it is simply necessary to de-afferent all roots of the sciatic nerve, so that when the peroneal nerve, which contains the motor neurones innervating the flexor muscle, tibialis anticus, is stimulated, the only possible central effect must be due to these antidromic impulses. The crossed extension reflex is readily evoked by stimulating the sciatic nerve in the opposite hind leg, whose dorsal roots need not be disturbed in this operative procedure.

Owing to the great difficulty experienced in obtaining a crossed extension reflex immediately after the disturbance to the spinal cord entailed in de-afferentation, the most satisfactory procedure is to sever the dorsal roots aseptically, and then to allow a week or so to elapse for recovery before performing the final experiment, in which the crossed extension reflex is evoked and the possible inhibitory effect of the antidromic impulses is sought.

Method. Operations were performed on a series of seven female cats under ether for de-afferentation of the left hind leg by cutting the dorsal roots of the lumbo-sacral plexus, central to the dorsal ganglia. All the cats survived, and in only one was there an infection, which luckily remained localized in a small area of the incision and did not penetrate to the cord. Of the seven, five cats gave significant results in the subsequent experiments, which were performed, in each case, a week to twelve days after the initial operation. One cat died during preparation for the final experiment, and one was found to be incompletely de-afferented; no records, therefore, were made of these two.

The recording apparatus—myograph, galvanometer and camera—was the same as that described by Forbes, Davis and Lambert (1930). For crossed extension stimulus (*E*), a Harvard coil with two dry cells was connected for tetanizing current (134 shocks per second, counting makes and breaks). This stimulus was graded by distance and angle of the secondary coil, the recorded angle being merely an approximate estimate. The threshold was found in each cat, and varying degrees of strength were used up to, and beyond, those causing maximal excursion of the myograph. For the antidromic stimulus (*I*) the rotary interrupter was connected in the primary circuit of a Bishop balanced coil and set at two or more frequencies during the experiment, usually in the range of 60 to 100 stimuli per second. Single makes and breaks were also used. The strength was varied by the angle of the primary coil, recorded on a dial, and controls of *I* alone were always made to determine the point at which direct spread of current to

the adjacent motor nerve caused contraction. In general, series were taken with weak, medium, and strong crossed extension, the antidromic stimulus being applied in varying strengths and at varying intervals during these different excitatory backgrounds.

The final experiments were prepared as follows. Under ether anesthesia, decerebration was performed with the Sherrington guillotine through the middle of the anterior colliculi. For recording crossed extension on the myograph, the soleus alone was used in the first experiment, the gastrocnemius and plantaris tendons being cut, but in succeeding experiments the whole muscle complex was used to insure a complete background. The anterior crural nerves, the psoas muscles, and the nerves to the hamstrings were always cut on both sides in order to eliminate extraneous movement. Fixation for myograph recording was effected by means of drills in the proximal and distal ends of the tibia.

The crossed extension stimulus (*E*) was applied by means of a pair of electrodes incorporated in a small hard rubber tube, described by Forbes, Davis and Lambert (1930), which was placed on the proximal portion of the cut right sciatic nerve. It was found in the second experiment that a vibration appeared on the string during *E*, due to a ground connection through the cat, and as this served as a signal for *E* without objectionable effect, the cat was purposely grounded in subsequent experiments.

For the antidromic stimulus (*I*) in the first experiment, electrodes in a slotted tube[1] were applied to the intact left peroneal nerve. Chlorided silver needle electrodes were inserted in the belly and tendon of the tibialis anticus muscle and led off to the galvanometer, in order that action currents from this muscle should demonstrate that the peroneal nerve was actually being excited. It was found, however, that contraction of the tibialis anticus caused a movement of the myograph lever in such a way as to obscure the evidence of presence or absence of true inhibition. In subsequent experiments, therefore, the peroneal nerve was cut near the knee and the stimulating electrodes were placed on the proximal portion, thus eliminating this confusing source of movement. In order to record the strength and timing of the inhibitory stimulus, leads to the galvanometer were henceforth connected in series with the secondary coil, a 2 ohm shunt being inserted in parallel with the string. The vibrations of the string due to *E* and *I* respectively were easily distinguished by the differences in size and frequency, the galvanometer thus serving as a signal for both. The general arrangement is shown in figure 1.

At the end of each experiment, certain procedures were undertaken as controls on conduction in the peroneal nerve. In the first animal, in which the nerve had been left intact, stimulation central to the tube electrodes

[1] An open slot enables the rubber tube to be applied to the intact nerve. Otherwise the nerve must be cut and threaded through the tube.

gave good contraction of the tibialis anticus muscle, showing that no block had occurred at the site of these electrodes; this was taken as evidence that impulses were being conducted centrally as well as peripherally. In cat 2, a second pair of tube electrodes was applied to the nerve, central to the pair already present. The galvanometer was connected to the proximal pair and action currents were demonstrated on stimulating at the original site, thus showing that the nerve was actually functioning antidromically. In

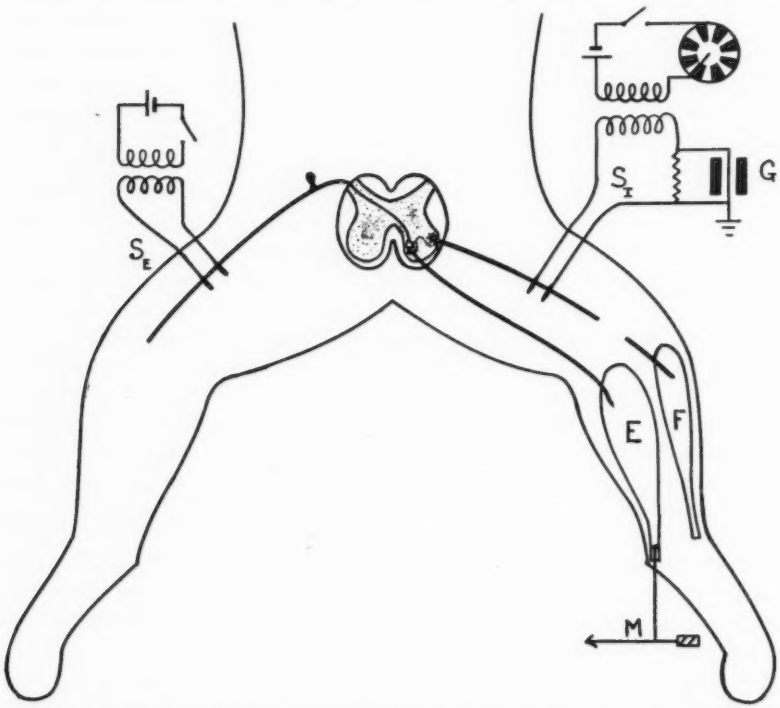

Fig. 1. Diagrammatic arrangement of preparation. *E*, extensor muscle; *F*, flexor muscle; *M*, isometric myograph lever; S_E, excitatory stimulating apparatus; S_I, apparatus for antidromic stimuli; *G*, string galvanometer in stimulating circuit to serve as signal.

the remaining experiments this procedure was carried out with the difference that the nerve was cut above the hip joint and isolated completely from the cat, the proximal portion being laid in a moist chamber to which the galvanometer leads were connected.

RESULTS. The post-operative condition and activity of the cats varied in detail, but in general the operated leg was held extended and scarcely

used in progression except in the case of no. 3. This cat's ability to walk was better than any of the others, for although she dragged the operated leg along on the dorsal surface of the paw and there was obvious ataxia, yet she actually put weight on it and managed to progress with comparatively little falling. Of the other cats, no. 1 always tumbled over on attempting to walk and made poor progress, no. 2 was active on three legs while nos. 4 and 5 merely pulled themselves along by the fore paws with no attempt to use the hind quarters at all.

Sensation was found absent over the whole left hind leg in all except the first cat in which there was a questionable small area on the medial aspect of the heel. Autopsy in this case revealed the left L4 rootlets to be intact and a small strand of left L5 apparently uncut. Autopsies in the rest showed the left hind leg to be completely de-afferented. In nos. 4 and 5 injuries to the cord were found, which probably explain the post-operative conditions, but do not affect the results of this particular investigation, since in all cases function of the anterior roots was demonstrated by crossed extension.

The final experiments showed varying degrees of crossed extension, but this was definitely present in all. Thus there was an excitatory background adequate to reveal any inhibitory effect which might be evoked by the antidromic impulses. In a survey of several hundred observations we find no evidence of any such inhibitory effect.

Certain individual differences in the myograms of different cats were noted. In some the onset of contraction was comparatively abrupt and the contractile tension well sustained during stimulation. In others the gradual onset of contraction, or recruitment, characteristic of the crossed extension reflex, was a conspicuous feature. In most of the observations a considerable degree of fluctuation in the plateau level occurred during the course of stimulation. In one preparation these fluctuations were great, and the resulting excitatory background was highly irregular. In all preparations cessation of stimulation was followed by a more or less distinct, transient increment of tension, similar to that found to result from light etherization (see Forbes, 1921). This rebound-like increment was not found in every observation, but occurred in a majority of those resulting from strong stimulation. Every animal in the series showed several instances of it. Presumably this effect is to be interpreted as evidence of a central inhibitory effect concurrent with the excitatory, but outlasted by the latter and probably due to the stimulation of inhibitory afferent fibres (cf. Forbes, 1921; Forbes, Davis and Lambert, 1930). The regularity of its appearance in this series may be a consequence of de-afferentation of the extensor muscle under observation, and the resulting loss of stretch reflex (cf. Fulton, 1926, chapters 15 and 16).

In a few instances the antidromic stimuli consisted of single induction

shocks. Usually, however, sustained stimulation by means of the rotary interrupter was employed. The excitatory stimulus was applied and maintained from 3 to 15 seconds. At various stages during the rise of contractile tension, or more commonly after it had become well established, the antidromic stimuli were applied, sometimes briefly and sometimes for several seconds. In many cases the antidromic stimuli were applied in two or three successive groups with intervals of a second or two between them, in order to afford a better opportunity for correlating changes in contractile tension with the application and withdrawal of these antidromic stimuli.

Examples of the observations are shown in figure 2. In a large majority of instances there was no suggestion of correlation between the fluctuations in contractile tension and the antidromic stimuli. In a few instances a distinct dip in the plateau of contractile tension appeared immediately after application of the antidromic stimuli (see fig. 2D), but these invariably occurred in preparations in which control observations with the excitatory stimulus alone showed similar dips or fluctuations from time to time. In a large number of records with such a fluctuating base line it is almost certain that a haphazard dip will sometimes be so timed as to appear correlated with the antidromic stimulus. Cases in which this occurred are offset in the same preparation by many instances of no change at all or of an actual increase in tension following the application of the stimulus. Certainly even with the strongest stimuli applied antidromically, nothing resembling real inhibition was observed. A careful survey of the entire series leads clearly to the conclusion that the antidromic impulses had no effect on contraction.

Discussion. We may conclude from our observations that backfiring antidromic impulses into the flexor motor neurones does not cause inhibition of the extensor center, and thus fails to reveal the inhibitory effect which has been tentatively ascribed to the side-fibrils of Golgi.

Although the results were negative and completely failed to demonstrate the hypothesis of inhibitory function in the side-fibrils, it is still conceivable that this function normally exists, but that for some reason the antidromic impulses failed to produce the effect which occurs when impulses are set up in the normal way through the reflex arc. For example, one might suppose that the distribution of thresholds of adjacent points in the conducting path is such as to permit excitation of the side-fibrils by the impulse entering the motor neurones through the dendrites, but not by the antidromic impulse approaching through the axons. There might conceivably be a clue to such a mechanism in the property of the reflex arc which provides the condition for irreversible conduction. The familiar fact that the reflex arc will not conduct impulses from the motor into the sensory neurones has been ascribed to some property of the synapse. It has been suggested

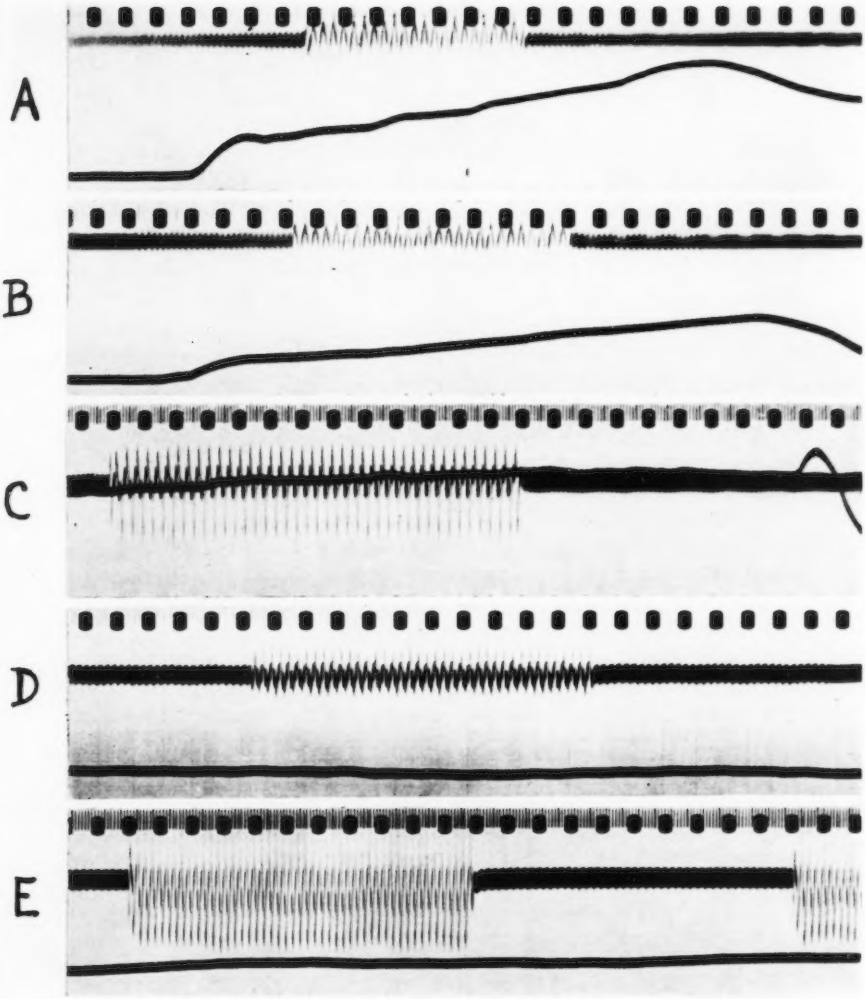

Fig. 2. Records showing failure of antidromic reflexes in flexor motor neurones to inhibit crossed extensor reflex.

The upper line in each shows string galvanometer record; fine oscillations recording excitatory stimuli, larger oscillations, antidromic stimuli. Lower and sharper line, record of isometric contraction. Time in 0.01 second intervals, shown by narrow bands between perforations at the top of each record.

A. and B. January 27, 1932. Records showing entire course of brief observation.

C. February 8, 1932. Shows the last part of prolonged period of reflex excitation with a series of antidromic stimuli.

D. and E. February 17, 1932. Antidromic stimuli during course of weak extensor reflex.

A. and C. both show rebound-like increment of tension on cessation of excitatory stimulation.

that this might be due to the distribution of chronaxies on the two sides of the synapse (Lillie, 1914; Forbes, 1922). It might be more simply explained by a distribution of thresholds, if we assume that the threshold in the motor neurone is sufficiently low to render the action current in the afferent neurone an adequate stimulus, while the threshold in the afferent fibre is so high that the action current in the motor neurone on antidromic stimulation fails to excite it. Since the side-fibril is a part of the motor neurone, the application of this principle to render it selectively excitable by impulses approaching from the dendrite and not to impulses approaching from the axons is highly improbable, but still conceivable.

Other conceivable ways of explaining the failure of our experiments to show positive results might be found through the assumption of a special rhythm of impulses in the side-fibril, necessary to produce the inhibitory effect normally resulting from the disturbances approaching through the synapse, but not reproduceable by antidromic stimulation. All these possibilities appear to us far-fetched and improbable, but are mentioned as loopholes through which an argument might be raised for the retention of the hypothesis of the inhibitory function of the side-fibrils. We conclude that while our evidence does not absolutely disprove this hypothesis, it renders it extremely improbable.

It is also possible that the side-fibrils are concerned with inhibition in a different manner from that indicated in the hypothesis as outlined. They may receive inhibitory influences directly from afferent paths,—a supposition which is in harmony with von Lenhossék's observation that the side-fibrils terminate in close proximity with collaterals from the posterior roots. Thus the afferent neurone which excites the flexor center would send impulses through the collateral to the side-fibril of the extensor motor neurone, where the inhibition of that neurone would in some way be effected. Our experiments furnish no evidence for or against such a mechanism. If we should assume this receptive rôle of the side-fibrils, it would still remain to explain how they could perform the function of inhibition.

Whatever the rôle of the side-fibrils, we are confronted by the question whether we may find a way of explaining reflex inhibition in terms of the properties of peripheral nerve impulses, without recourse to special functional capacities in the center, which have no counterpart in the peripheral neuro-muscular unit.

Eccles has shown that an antidromic impulse cuts down or removes preformed central excitatory state, whatever that may be, at the synapse. This effect bears an obvious resemblance to inhibition. The question arises, can the two effects be essentially the same?

In spite of the differences in time relations and summation properties emphasized in the comparison of reflex function with nerve-trunk conduc-

tion, the recent discussions of Eccles and Sherrington (1931) and of Adrian and Buytendyk (1931) have shown an inclination to interpret the activity in the center in terms of the membrane theory, as a depolarization, thus viewing it as fundamentally akin to nerve-trunk conduction. Following the same thought, summation, which is an essential feature of reflex function, may be regarded as a cumulative effect of action potentials upon the structure whose threshold must be reached before conduction can progress farther. Eccles and Sherrington (1931b) have contrasted the view that summation depends on a progressive depolarization of the membrane with the view that persistence of the action potential causes a cumulative effect of successive impulses. Conceivably a complete understanding of the mutual relation between the action potential, as the exciting agent, and the depolarization of the membrane where its effect is exerted, would lead to the identification of the two interpretations as being essentially the same. In the case of summation due to simultaneous impulses in convergent paths, we may assume, as a working hypothesis, that the combined action potentials exert more depolarizing effect on the tissue at or beyond the point of convergence than does the action potential in a single path alone.

That the antidromic impulse in Eccles's experiments does not summate with afferent impulses in producing an excitatory effect, but, on the contrary, obliterates whatever excitatory effect is already there, may perhaps be most simply explained on the principle of collision. It is well known that if two impulses are made to travel in opposite directions in a single nerve fiber, when they collide they block each other, for the reason that each is followed by a refractory period. The block in collision, then, depends on the placing of a refractory phase in the path of each impulse. The reason why the antidromic impulse exerts a blocking effect at the synapse instead of summation with excitatory effects, may be due to the opposed direction of travel in the conducting path.

The superficial resemblance between the antidromic block in the center and reflex inhibition, suggests the question whether inhibition could also be explained on the principle of collision. This would be another way of invoking refractory phase; the essential feature in the Wedensky effect, which has been proposed as a possible clue to reflex inhibition. But the collision principle has the advantage that it does not depend on the critical adjustment of rhythm to the rate of recovery, which is itself a variable, a weak point in the hypothesis based on the Wedensky effect. If reflex inhibition could be explained on the basis of collision due to the anatomical arrangement of the fibers whereby the inhibitory afferent impulses are conducted into the synaptic region, it would be a most desirable simplification, since it would involve no functional property other than the well-known refractory phase. We have not been able to think of any arrangement whereby the effect of collision could be obtained without introducing

the conditions for reinforcing excitatory effects by the inhibitory afferent impulses, and this is contrary to fact. We believe, however, that such an explanation of inhibition is not wholly ruled out, for some property in the central structures, as yet unknown, may conceivably serve to confine the effect of inhibitory impulses to collision and prevent any summation with excitatory impulses.

Recent work of Eccles and Sherrington (1931c) on the temporal course of inhibition appears difficult to reconcile with any hypothesis which invokes refractory phase as the basis of inhibition, whether in the form of Wedensky effect or collision. The discussion of their experiments deals with the relations of central excitatory and inhibitory states ("c. e. s." and "c. i. s."). They show that a single afferent volley produces on the contralateral flexor center an inhibitory effect which rises to a maximum in about 80 σ and subsides again in a similar time. They ascribe this relatively long duration to two distinct factors,—temporal dispersion of impulses in paths involving different degrees of delay, and persistence of the resulting c. i. s. at the synapse of the motor neurone. One argument for this differentiation is that summation of central inhibition endures too long to be explained as facilitation between afferent volleys in the internuncial neurones. But the evidence cited in support of this is derived from the flexion reflex, whose reflex time is much briefer than that of the crossed extension reflex, and since the inhibitory effect under consideration is part of the crossed extension reflex, it is reasonable to expect facilitation to endure longer in this more complex path than in the simpler flexion reflex path. In this connection it is of interest to note the close agreement of reflex time for inhibitory impulses crossing the cord, found by Eccles and Sherrington (1931c, p. 105) and for the excitatory impulses in the same reflex, found by Forbes and Cattell (1924, p. 145).

Another argument of Eccles and Sherrington for this distinction between the nerve impulse and c. i. s. factors in the duration of inhibition is that an excitatory volley neutralizes some of the effect of central inhibition on a subsequent excitatory reflex. The brevity of the effect is cited as evidence against its interpretation as an inhibition of the internuncial neurones in the inhibitory path, and therefore as favoring the view that c. e. s. acts directly on c. i. s. at the motor neurone. The evidence is indeed favorable to that view, but can hardly be deemed a proof of the precise nature or locus of the neutralizing effect.

Clearly the facts now known render it impossible to explain all the phenomena of reflex inhibition by any such simple application of the Wedensky effect as was first proposed by Lucas (1911). Provision must be made for some mechanism whereby central inhibitory effects, arising from a single afferent volley, can endure far longer than a single refractory period. Complexity in the central structure may be invoked to account for repetitive

impulses in certain conducting paths. Altogether the accumulating evidence seems to render any interpretation based on the Wedensky effect increasingly improbable. We would contend, however, that as yet too little is known about the real distribution of conducting paths in the gray matter, and of the activities which occur in them, to enable anyone to pass final judgment on the provisional hypotheses which have been offered to explain reflex excitation and inhibition. A more complete knowledge may yet reveal a way to interpret reflex excitation as a special case of the same kind of activity observed in the peripheral nerve impulse, and inhibition as a consequence of refractory phase, either through some arrangement which provides for collision or through some other arrangement as yet unknown.

SUMMARY

1. The side-fibrils of Golgi have been suggested as a possible mechanism for reciprocal inhibition in spinal reflexes. If the side-fibrils given off by the flexor motor neurones conduct impulses which exert an inhibitory effect on the extensor center, this effect should appear when these flexor motor neurones are excited by antidromic impulses.

2. The sciatic nerve of the cat was completely de-afferented, subsequently the animal was decerebrated, and with the crossed extension reflex as a background, the peroneal nerve was stimulated in order to set up antidromic impulses.

3. In no case did this procedure inhibit the crossed extension reflex, for no significant change in the contractile tension could be correlated with the antidromic stimuli.

4. It is still possible that the side-fibrils are involved in inhibition, perhaps by receiving afferent impulses and conveying an inhibitory effect to the neurones whence they arise.

5. All the animals in this series manifested an increase in contractile tension on cessation of the crossed extensor stimulus, resembling the effect previously recorded under light etherization, and suggesting the activity of an inhibitory reflex during the application of the stimuli.

6. A resemblance is noted between reflex inhibition and the blocking of reflex excitation by antidromic impulses in Eccles' experiments, and it is suggested that a way may be found to explain inhibition as a block dependent on the refractory phase placed in the path of the impulse, as in the case of two impulses colliding in a single fiber.

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OSMOTIC RELATIONS BETWEEN BLOOD AND BODY FLUIDS

II. The Osmotic Relation of Blood and Gastric Juice[1]

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In recent years there has been a growing realization that most of the glandular secretions of the body are approximately isotonic with the blood. An example of such type of investigation is the work of Gamble and McIver who examined gastric juice (1928a), pancreatic juice (1928b), and bile (1928b). They found that the total ionic content of these fluids was approximately the same as that for the total fixed base of the blood. In their study upon the gastric juice, Gamble and McIver observed that practically all the anion of this secretion was represented by the Cl ion. The chloride concentration was approximately 155–160 milli-equivalents (*m eq.*), a figure very close to the accepted value for the total fixed base of the blood. Such agreement between the total anion content of one fluid and the total cation content of another indicates that they are approximately isotonic.

For purpose of differentiation, let us consider such an experiment as a static one, in which a "normal" figure for gastric juice was compared with an *average* value for blood. From such a type of investigation two possible conclusions could be drawn: 1, the gastric juice is a fluid of fixed composition and this composition is such that it is approximately isotonic with the blood, or 2, there is a physico-chemical equilibrium between blood and gastric juice as a result of which, even in the presence of changes, the two fluids are isotonic in obeyance to certain physico-chemical laws. The second conclusion could be proven if one were to study these two fluids in a dynamic type of experiment. In other words, if one should produce significant changes in the ionic content of the blood, there should be observed corresponding and parallel changes in the concentration of the ions of the gastric juice. This paper is a report of such experiments.

Methods. In a study of a physical equilibrium between two fluids of widely different composition in which one is mainly interested in the toni-

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cities of the two relative to each other, the most valuable determinations that can be made are with respect to their osmotic pressures. Such data give information concerning the total ionic and molecular concentrations of the substances under examination. In this study osmotic pressure determinations on both blood and gastric juice were made using the Hill vapor pressure method (1930). It is unnecessary to give the manipulative details of this method inasmuch as they have been published in full by Margaria (1930). The method is rapid in execution. We have found the maximum experimental error to be about $\pm$ 0.5 per cent. In addition this procedure is of great advantage in that all errors due to the escape of blood gases are eliminated. The gastric juice was also analyzed chemically for its total Cl content, the method of Van Slyke (1923) being employed.

EXPERIMENTAL PROCEDURE. In a study of gastric juice one must bear in mind that this fluid, as obtained for examination, is the product of the activity of at least two sets of glands. In this investigation our interest was centered on the secretory products of the parietal cells, inasmuch as the inorganic constituents of the gastric juice account for most of its osmotic pressure. The presence of large quantities of organic substance would only serve to complicate the experiment. Consequently gastric juice was obtained in response to a histamine stimulus. It has been shown by Vineberg and Babkin (1931) and Gilman and Cowgill (1931a) that histamine acts exclusively on those cells concerned with the elaboration of the inorganic constituents of the gastric juice.

Pavlov pouch dogs were used in this study. Histamine (0.2 mgm. per kilo) was administered subcutaneously. After the injection of the drug, at a time when the gastric juice was flowing at its maximum rate, a sample was collected for analysis. As simultaneously as was possible, a blood sample was drawn from the femoral artery. The blood was defibrinated by whipping with glass beads, and the osmotic pressure determination made upon this defibrinated whole blood. The determination was conducted almost immediately after the sample was obtained. After samples were procured from the normal animal, large quantities of hypertonic saline were administered by stomach tube. Blood and gastric juice samples were obtained in exactly the same manner as outlined above 90 and 180 minutes after the salt was administered. The animal was then offered water *ad lib.* and the final group of samples obtained 90 minutes after the water had been consumed.

In another series of experiment the procedure was reversed. After obtaining a normal sample of blood and gastric juice, large quantities of water were administered by stomach tube. Again samples were collected 90 and 180 minutes after the administration of water. Hypertonic saline was then given, and a final sample taken 90 minutes after the animal had received salt.

TABLE 1

DOG EXPERIMENT	EXPERIMENTAL PROCEDURE	OSMOTIC PRESSURE—BLOOD	OSMOTIC PRESSURE—GASTRIC JUICE	CHLORIDE GASTRIC JUICE
		m. eq.	*m. eq.*	*m. eq.*
E15	200 cc. 5% saline	158.9	163.6	162.7
		168.6	169.1	169.0
		169.1	171.9	170.4
		162.8	160.7	162.0
Y17	200 cc. 5% saline	157.2	149.2	149.0
		174.3	160.6	164.0
		174.1	163.2	163.4
		155.9	149.2	150.2
E19	200 cc. 5% saline; some vomited	167.1	167.7	165.8
		178.1	175.2	174.4
		175.7	174.0	173.4
		161.7	162.8	163.2
Y112	200 cc. 5% saline; vomited?	157.5	157.4	152.0
		162.0	156.3	158.4
		177.6	168.0	166.0
		160.0	155.9	154.4
E114	200 cc. 5%; vomited; 200 cc. 4% saline	161.5	162.5	160.0
		175.8	162.9	164.0
		175.0	172.2	
		160.1	162.3	162.4
E119	250 cc. 4% saline	161.5	161.0	159.6
		165.3	162.7	162.0
		168.7	165.6	164.0
		160.0	158.9	160.0
P123	550 cc. 3% saline	147.2	147.7	150.4
		164.7	157.4	157.6
		170.6	155.4*	155.6*
		161.0	153.8	153.2
E121	1400 cc. water	156.6	161.3	160.8
		137.8	137.7	139.6
		146.5	145.0	145.2
	No salt	147.7	151.1	152.0
Y121	1900 cc. water	158.6	153.0	154.4
		137.3	139.5	142.4
		138.1	142.1	146.8
		151.1	150.9	151.6

* Large amount of mucus present in sample.

TABLE 1—*Continued*

DOG EXPERIMENT	EXPERIMENTAL PROCEDURE	OSMOTIC PRESSURE—BLOOD	OSMOTIC PRESSURE—GASTRIC JUICE	CHLORIDE GASTRIC JUICE
		m. eq.	*m. eq.*	*m. eq.*
E126	1550 cc. water	146.3	156.1	153.2
		121.2	136.1	136.0
		119.5	136.6	137.2
		133.2	148.2	151.2
Y126	1700 cc. water	143.1	152.1	154.8
		128.0	139.0	142.4
		121.3	138.0	140.4
		135.7	145.9	151.2
E128	1800 cc. water	156.2	164.1	162.2
		146.2	156.9	154.8
		149.2	156.2	155.2
		161.2	169.5	167.8
Y128	2000 cc. water	153.8	151.3	150.8
		134.4	144.1	142.2
		135.0	142.1	143.6
		158.1	160.3	161.8
T130	1250 cc. water	153.0	153.3	154.2
		137.4	140.3	136.0
		133.8	135.0	135.4
		142.2	145.3	146.6
T22	1500 cc. water	153.5	158.0	157.6
		130.4	139.1	141.6
		135.5	144.8	144.0
		160.5	161.2	162.2
T24	1500 cc. water	146.3	160.7	158.0
		122.2	143.1	147.2
		129.5	144.1	145.2
		161.3	167.3	166.8
B24	2500 cc. water	152.5	153.3	156.0
		140.3	145.6	142.2
	Vomited about 1500 cc.	139.8	146.0	144.6
		165.5	168.0	167.2
P26	2000 cc. water	156.1	148.9	150.8
		136.9	136.8	140.0
		136.4	136.0	138.8
		147.2	144.6	145.6

TABLE 1—*Concluded*

DOG EXPERIMENT	EXPERIMENTAL PROCEDURE	OSMOTIC PRESSURE—BLOOD	OSMOTIC PRESSURE—GASTRIC JUICE	CHLORIDE GASTRIC JUICE
		m. eq.	*m. eq.*	*m. eq.*
P28	1750 cc. water	153.1	152.1	151.0
		133.5	139.6	140.0
		136.3	140.2	140.0
		157.1	147.5*	148.2*
B29	1750 cc. water	151.1	146.2	145.6
		130.9	138.1	142.2
		132.7	145.1	144.4
		159.9	162.0	162.2
T29	1700 cc. water	151.1	158.7	160.0
		131.1	139.3	138.2
		129.9	144.4	143.2
		157.6	161.9	161.7
B211	1750 cc. water	156.1	144.3*	
		133.0	134.9	
		135.0	138.8	
		163.6	165.0	
P211	1750 cc. water	154.7	162.2	
		138.3	120.5*	
		140.0	144.8	
		156.6	157.2	
B216	1750 cc. water	155.4	159.8	
		135.4	137.1	
		140.2	145.4	
		160.7	168.2	

RESULTS. A total of 96 simultaneous observations on blood and gastric juice was made upon 5 dogs. Table 1 is a complete report of the data obtained. The osmotic pressure of the fluids under examination is expressed in milli-equivalents of an osmotically equivalent NaCl solution. Inspection of the data reveals the following significant correlations.

The experiments are summarized in groups of four figures. The first figure of the group represents data obtained from "normal" samples; the second and third figures represent the values obtained 90 and 180 minutes after the experimental procedure indicated; the last figure shows that obtained after the original procedure had been reversed, i.e., if the animal originally received large quantities of hypertonic saline, the final values given represent the condition of the blood and gastric juice after the animal had been offered water.

Relation between the osmotic pressure of blood and gastric juice. It is evident from an inspection of the data in the table that there is a definite

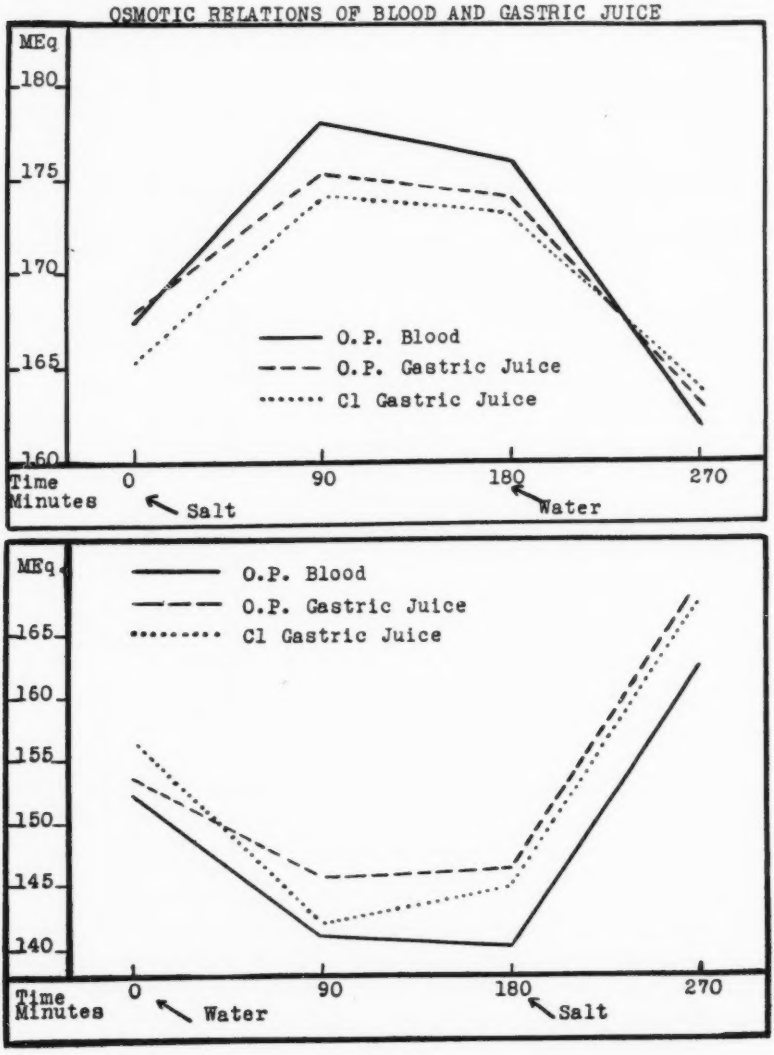

Fig. 1

relationship between the osmotic pressure of the blood and gastric juice. In every experiment, with an increase or decrease in the osmotic pressure

of the blood produced by either saline or water administration, there is observed a corresponding change in the osmotic pressure of the gastric juice. Graphic illustrations of this point are presented in figure 1. Statistical treatment of the data shows the coefficient of correlation between the osmotic pressures of these two fluids to be $\pm 0.865 \pm 0.017$. The contention that the blood and gastric juice are in a physical equilibrium is supported by such a significant correlation coefficient. Further discussion of these respective osmotic pressures is given later in this paper when other findings of interest have been described.

Relation between the osmotic pressure of the gastric juice and the total chloride content of the gastric juice. As has been previously stated the figures representing the osmotic pressure of the gastric juice are expressed in milli-equivalents of an isotonic NaCl solution. A comparison of the figures so obtained by physical measurement with the chloride values of the gastric juice yielded by chemical analysis shows a striking agreement. The coefficient of correlation between these two series of values is $\pm$ 0.981 ± 0.0003, whereas the average deviation between the two groups is 1.5 m. eq. and the standard deviation only 1.96 m. eq., a difference only slightly greater than that of the errors of the experimental methods.

The significance of this close correlation between the osmotic pressure and the total chloride content of the gastric juice is worthy of consideration. Our previous correlation indicates that the osmotic pressure of the gastric juice is controlled and regulated to a very high degree, if not entirely, by that of the blood. Further, inasmuch as the Cl ion represents practically all of the osmotically active anion of the gastric secretion, it follows that the osmotic pressure of the blood is the primary factor which determines the chloride concentration of the gastric juice. Moreover, the total chloride concentration of the gastric juice limits the maximum acidity which this fluid can attain. Therefore it is reasonable to claim that the osmotic pressure of the blood plays the primary rôle in the limitation of gastric acidity. Pure parietal secretion, obtained by the action of histamine, has been observed in this laboratory (Hollander and Cowgill, 1931) and by numerous other invéstigators to have an acidity closely approximating the theoretical maximum, namely, that acidity at which all the chloride of the gastric juice is present as HCl in concentration approximately isotonic with the blood. In the normal course of events, however, when this pure parietal juice is diluted with admixtures of food, mucus, and the secretions of other glands of the stomach, much higher pH values are to be expected.

Relation between the osmotic pressure of the blood and the rate of secretion of gastric juice. An interesting correlation that was brought out upon examination of the data at the conclusion of our studies was that indicating a relationship between the osmotic pressure of the blood and the rate of

formation of gastric juice. This relationship is graphically represented in figure 2. In brief, it may be said that for a given stimulus the rate of formation of gastric juice was found to be inversely proportional to the osmotic pressure of the blood. The explanation of this observation is believed to lie in the changes in permeability of the capillary bed produced by marked physical changes in the circulating fluid.

Discussion. Although in general it may be said that the gastric juice and blood are approximately isotonic, there are instances, as can be seen from the table, where the respective osmotic pressures of these fluids vary appreciably. Qualitatively, in every instance an increase in the osmotic

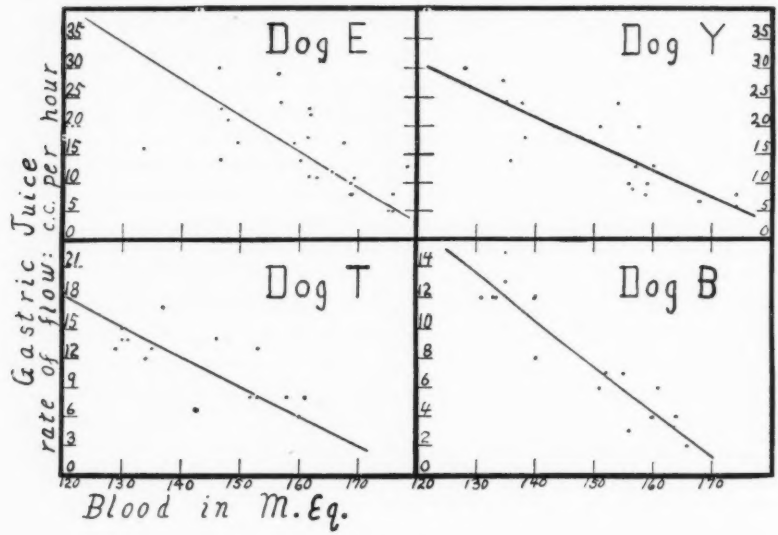

Fig. 2

pressure of the blood was accompanied by an increase in the osmotic pressure of the gastric juice. The same parallelism is observed when one considers decreases in the osmotic pressures of these fluids. However, these changes are not always of exactly the same magnitude. In general, after the administration of salt the osmotic pressure of the blood tended to be higher than the osmotic pressure of the gastric juice. On the other hand, after the administration of water there was a marked tendency for the gastric juice to be hypertonic. It is difficult to explain these discrepancies. A partial explanation may lie in the variations observed in the secretion rate of the gastric juice. After the administration of salt this rate was markedly diminished. When the rate of secretion of the juice is low, the

proportion of "extra-parietal" substances, such as mucus, tends to be greater than when the juice is flowing freely. These large amounts of organic material lower the osmotic pressure of the fluid. A few isolated instances, in which there was an exceptionally large quantity of mucus in the gastric sample, are marked in table 1. It can be seen that in such cases the osmotic pressure of the sample is abnormally low. This may be an explanation for the tendency for the blood to be hypertonic to the gastric juice after the administration of saline. However after large quantities of water are given the relative tonicities are reversed. This may be partially explained by the fact that the gastric juice under such conditions is flowing very rapidly. In addition, it must be remembered that one is comparing the osmotic pressure of gastric juice, supposedly equilibrated with gastric arterial-venous blood, with the osmotic pressure of systemic blood obtained from the femoral artery. It is difficult to state specifically what changes are taking place in the gastric blood as the stomach glands extract from it the substances necessary for the formation of their secretion. It is reasonable to suppose, however, that a much closer correlation of the osmotic pressures of these fluids could be obtained if one were to compare the relative tonicities of gastric juice and gastric venous blood.

In a previous communication (1931b) the authors suggested a possible mechanism for this physical equilibrium. The results presented in this paper constitute a confirmation of the hypothesis that the chloride concentration of the gastric juice is determined chiefly, if not solely, by the osmotic pressure of the blood. It is suggested that this osmotic equilibration takes place in the lumen of the gastric glands. It is believed that gastric Cl is secreted by the parietal cells as dilute HCl. A consideration of the mechanism of its formation is not pertinent to this discussion. It is assumed, however, that the acid is secreted in very dilute solution because staining techniques have not revealed any great acidity in the parietal cells. In view of the wide difference in concentration of blood and gastric chloride, it must be assumed that the chloride ion, after having been secreted into the lumen of the gland, is unable to return to the blood stream: the cells lining the glandular lumen must be impermeable to this ion. Therefore, equilibration is most likely obtained by a passage of water from the glandular lumen into the circulation until equilibrium is established. By the time the secretion leaves the tubular glands it has an osmotic pressure and chloride concentration defined and regulated, as indicated above, by the osmotic pressure of the blood.

SUMMARY AND CONCLUSIONS

A series of simultaneous observations has been made upon the osmotic pressure of dog's blood and gastric juice. They were found to be practically isotonic, with the osmotic pressure of the gastric juice almost entirely

a function of its chloride concentration. The osmotic pressure of the blood was widely changed by the administration of either hypertonic saline or water. Variations in the osmotic pressure of the blood were associated with parallel changes in the osmotic pressure of the gastric juice. In view of this physical equilibrium the opinion is formed that the osmotic pressure and chloride concentration of the gastric juice are regulated by the osmotic pressure of the blood, a water exchange across a membrane impermeable to the Cl ion being the fundamental mechanism involved. An inverse relationship between the osmotic pressure of the blood and the rate of formation of the gastric juice was also observed.

We wish to express to Dr. Ethel Burack and Mr. Gustav Stoehr our appreciation of their assistance rendered in certain of the experiments.

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THE EFFECT OF ADRENALIN INSTILLATION ON THE IRIS OF NORMAL ANIMALS

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It has been repeatedly stated that the instillation of adrenalin into the conjunctival sac of a normal animal has no effect on dilatation of the pupil (i.e., the radial contraction of the iris). Radziejewsky (1898) was the first to report that in patients the instillation of adrenalin into the eye produced no change in the size of the pupil. This observation was later confirmed by Lewandowsky (1899), Boruttau (1899), and Loewi (1908) on cats, by Wessely (1905) on dogs, and by Meltzer and Meltzer-Auer (1904) on rabbits. Koppányi and Sun (1926), on the other hand, state that in normal rats and mice the instillation of adrenalin into the conjunctival sac produces a very slight dilatation of the pupil. They were, however, unable to obtain any effect on the avian iris.

After removal of the superior cervical ganglion the iris becomes much more responsive to adrenalin. Numerous investigators have observed that under this condition its instillation into the eye causes a distinct mydriasis. A similar adrenalin mydriasis can be brought about by conditions other than removal of the superior cervical ganglia. For example, in certain pathological states in which an alteration of the continuity of the epithelial layer of the conjunctiva takes place, adrenalin instillation into the eye causes a widening of the pupil (Poos, 1927). Furthermore, it has been demonstrated experimentally (Wessely, 1905) that if adrenalin is injected under the conjunctiva in a normal animal a marked mydriasis occurs. In view of these facts, it seemed possible that in normal animals, also, a dilatation of the pupil might be obtained with adrenalin instillation, if conditions were made more favorable for its absorption, e.g., by prolonged instillation or by removal of the nictitating membrane.

METHODS. Normal cats were used for most experiments, although a few observations were made on rabbits. The latter animals were found unsatisfactory because of the difficulty in making simultaneous readings on both eyes.

Measurements of the pupils were taken by means of a millimeter scale

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held close in front of the eyes and at right angles to the vertical pupillary opening. The eyes were invariably subjected to a standard illumination. The adrenalin solutions for instillation were made up fresh for each experiment; 1:10,000 and 1:2,000 dilutions were most constantly employed. The solution was instilled in only one eye; the other was used as control. The instillation was made by holding apart the eyelids and dropping in sufficient solution to flood the eyeball. Every few minutes the instilled solution was removed by wiping the eye with cotton and fresh drops added. The time during which the adrenalin solution was kept in contact with the conjunctiva varied from 5 to 30 minutes.

RESULTS. The instillation of a solution of adrenalin (1:10,000) for 5 to 10 minutes caused no modification in the size of the pupil. A slightly

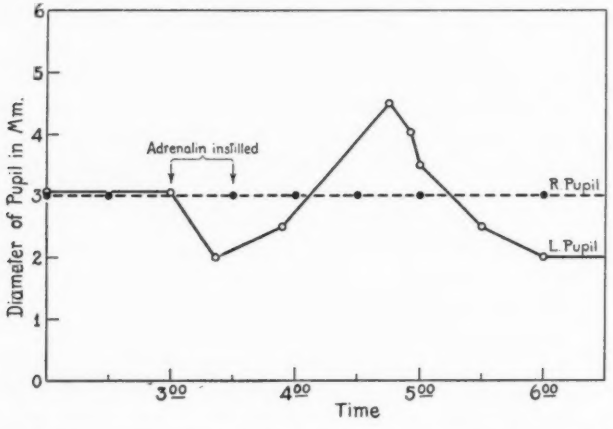

Fig. 1. Shows the contraction, dilatation and subsequent contraction of the left pupil produced by instillation of a 1:2,000 solution for 30 minutes.

longer period of administration in some animals produced a narrowing of the pupil. This contraction constantly occurred when a 1:10,000 solution was instilled for 30 minutes. The contraction observed in the instilled pupil is usually from 0.5 to 1.0 mm.; it appears in 15 to 30 minutes after the instillation and may persist for five hours. It is as clearly visible in dim as in bright light. The instillation of a stronger solution, 1:2,000 for 10 minutes, produced the same effect as the more dilute solution instilled for a longer time, namely, a contraction of the pupil. When, however, the 1:2,000 solution is administered for 30 minutes, the initial contraction of the pupil that appears after the instillation has been continued for 3 to 20 minutes, is, in the majority of animals, followed by a striking mydriasis. This mydriasis amounts to an increase of the width of the pupillary opening

by 1 to 3 mm.; it may persist for several hours and is frequently followed by a second contraction of the pupil (fig. 1). These observations of pupillary changes after instillation of adrenalin were made in 31 animals and the results were consistently similar to those described above.

It was found that in rabbits there was a marked response of the iris to instilled adrenalin. In this animal 4 or 5 drops of 1:1,000 solution administered for five minutes produced a marked widening of the pupil which was not preceded by contraction as in cats.

Some observations were also made on cats in which the nictitating membrane had been removed aseptically some days before the experiment, because Meltzer (1904) suggested that the membrane sweeping over the eyeball during the instillation might prevent the action of the drug. In these animals the effect on the iris of the instilled adrenalin was considerably increased. For example, a 1:10,000 solution instilled for 10 to 30 minutes caused a distinct widening of the pupil, sometimes preceded by contraction. In normal animals with membranes intact a dilatation was never observed with this solution of adrenalin and this time of instillation. Section of the sympathetic nerve in the neck, after removal of the nictitating membrane, further increased the reaction of the iris to instilled adrenalin, so that a solution of 1:10,000 administered for 2 minutes was sufficient to produce the mydriasis.

Since Schlossberg (1932) has shown that instillation of adrenalin into the eye, previously sensitized by removal of the superior cervical ganglion, increases the response of the iris to adrenin circulating in the blood, the question arose as to whether instillation of adrenalin into the normal eye would have the same effect. For this reason some experiments were done in which adrenalin was injected intravenously or the animals emotionally excited after one of the eyes had been previously instilled with adrenalin.

Adrenalin (1:2,000–1:25,000) was injected intravenously into normal cats which showed either contraction or dilatation of the pupil after previous instillation. In animals in which the instilled eye was showing contraction the intravenous injection produced an immediate and marked dilatation in both pupils. This was, however, more marked in the instilled eye (see fig. 2-A) and persisted for several minutes after the pupil of the non-instilled eye had returned to its previous diameter. Following the period of dilatation the pupil of the instilled eye again showed a contraction which gradually increased in magnitude from 0.5 to 1.0 mm. When adrenalin was injected intravenously into a normal animal having a mydriasis in one eye from a previous instillation of a 1:2,000 solution (the injection was made when the mydriasis of the pupil had nearly worn off), a striking dilatation occurred immediately in both pupils, but was much more marked in the instilled eye. The pupil of the control eye quickly returned to its normal size while that of the instilled eye remained strikingly dilated for more than 20 minutes (see fig. 2-B).

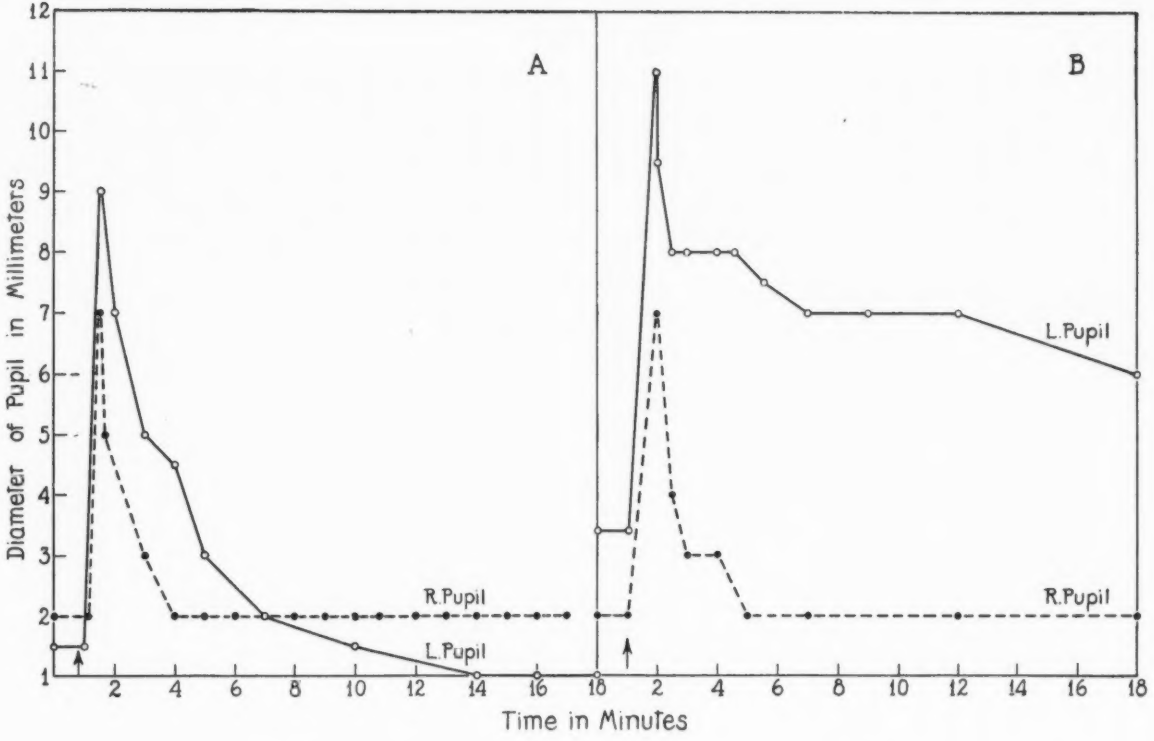

Fig. 2-a

Fig. 2-b

Fig. 2-A. Shows the effect on the pupils of intravenous injection of 1 cc. 1:2,000 adrenalin when the left pupil was in a contracted state from previous instillation of adrenalin.

Fig. 2-B. Shows the effect of intravenous injection of 1 cc. 1:10,000 adrenalin when the left eye was showing mydriasis from previous instillation of adrenalin.

Emotional excitement (by exposure to a barking dog) was produced in some of the normal animals in which one eye had previously been instilled with adrenalin. When the instillation had caused a contraction of the pupil, subsequent emotional excitement did not change the relative width of the pupillary opening between the instilled and non-instilled eyes. In an animal with the nictitating membranes already removed, however, emotional excitement increased quite noticeably the mydriasis which was produced previously by instillation of adrenalin. The failure of emotional excitement to cause an effect on the instilled eye in normal animals is possibly due to too small an amount of adrenin being secreted during excitement to have any action, since we found that quite strong solutions of adrenalin had to be given intravenously in order to produce an effect.

The greater action of adrenalin after removal of the nictitating membrane may be due to breaking the continuity of the conjunctival epithelium and thus leaving a region through which the drug can be more easily absorbed. Such an hypothesis is admissible, in view of the fact that various investigators have observed that certain pathological conditions which modify the permeability of the conjunctival epithelium cause an increased response of the iris to adrenalin. On the other hand, the nictitating membrane may prevent the action of instilled adrenalin on the iris merely by its physical action in sweeping over the eyeball, as was suggested by Meltzer (1904). A third possibility is that removal of the membrane produces a pocket between the eyeball and the eyelids, thus forming a receptacle for the instilled adrenalin and giving it a larger surface from which to act.

The observation that different amounts of instilled adrenalin have opposite effects on the muscles of the iris (small doses causing myosis, large doses mydriasis) are in accordance with the findings of Elliott (1905). He saw a marked constriction of the iris of the dog and goat when adrenalin was injected into the jugular veins. However, if a little adrenalin was injected into the carotid artery or into the anterior chamber of the eye, a mydriasis was observed.

SUMMARY

1. Adrenalin, if instilled for a sufficiently long time into the conjunctival sac of normal cats or of rabbits, exerts an effect on the muscles of the iris.

2. The effect of minimal doses of instilled adrenalin in cats is to cause a constriction of the pupil. Larger amounts of the drug produce a distinct mydriasis in the instilled eye which is in some cases preceded and followed by a period of contraction (see fig. 1).

3. Previous removal of the nictitating membrane in cats greatly increases the effect of adrenalin instilled into the otherwise normal eye.

4. Intravenous injection of adrenalin into a normal animal showing contraction of the pupil of one eye from previous instillation of adrenalin

from previous instillation of adrenalin.

...ct of intravenous injection of 1 cc. 1:10,000 adrenalin when the left eye was showing mydriasis

produces a more striking and more persistent mydriasis in that eye than in the normal one (see fig. 2).

5. Intravenous injection of adrenalin into a normal animal showing dilatation of one pupil from previous instillation produces in the instilled eye a more marked mydriasis than in the other eye. In this case the mydriasis of the instilled eye remains for a much longer time after the normal pupil has reached its original diameter than in the experiment described above (see fig. 2-B).

In conclusion, we wish to express our thanks to Dr. W. B. Cannon for his kindly interest and helpful criticism.

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THE CORTICAL RESPONSE TO STIMULATION OF THE OPTIC NERVE IN THE RABBIT

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The following description of the response of the optic cortex to stimulation of the optic nerve presents the first stage of a projected study of the entire optic tract in terms of the electrical activity of its various elements. Our basic assumption is that the functioning of each element of such a nervous pathway will be accompanied by an electrical potential; that this potential is a valid and potentially quantitative index of such activity; and that of the concrete signs of cell activity detectable, action potentials can be recorded most precisely, and with the minimal disturbance of normal activity.

When the optic nerve of the rabbit is stimulated directly by single electrical shocks, the retina having been cut away, there can be recorded from the surface of the optic cortex a rather specific series of electrical potential waves, presumably indicating the response of various neurones of the optic pathway. There are typically five of these waves, arising at intervals of about one-fifth second. A single stimulus applied to all the fibers of the optic nerve is hardly comparable, from the point of view of the sensation to be expected, to the complicated pattern of stimuli which the retina itself must deliver to the optic nerve fibers in any given visual act. Our procedure has one advantage however, in that the response is simpler to analyse if the retinal complications are out of the picture, and if the responses of all the elements in parallel occur at the same time. We are taking as a working hypothesis the assumption that if we can trace first a simple impulse through the sequence of nervous elements that must make up the whole visual pathway, detecting its course and its time of arrival at various points, we will presumably have data that can be applied to the interpretation of more complex responses; quite as, from the anatomical side, a study of neurones contributes to a knowledge of nervous pathways made up of many neurones. The only claim we make for these results is that they give us information as to one way in which the optic pathway *can* function,

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not that they tell us how it always *does* function in the intact animal. That the succession of electrical potentials following a single stimulus signalizes the activity of successive neurones along the visual pathway, probably successive layers of cortical cells, seems the most plausible explanation of these records, and some evidence for this inference will be presented in due course. We are certain however, that we have not detected the activity of all the elements involved. We infer, that is, that these cells responding are *among* those whose activity results in vision; but we wish to avoid for the present any interpretation of psychological function parallel to the physiological functioning here studied. There is one general fact, however, that may be worthy of consideration in what follows. While the results from over fifty rabbits successfully recorded show complete agreement in the general type of responses obtained, certain details of the records vary considerably, and as yet unpredictably, both from animal to animal and in the same animal under different conditions. This is a reasonable result merely from the physiological complexity of the pathway involved, and the corresponding psychological variability of sensory phenomena may be supposed to correspond similarly to a basic physiological complexity.

TECHNIC. Rabbits have been prepared in a variety of ways with uniform results. Operations were performed under light general ether, amytal or magnesium sulfate anesthetic, and under local novocaine. At a concentration of anesthetic that just keeps the animal quiet, amytal seems to depress the cortical response to the greatest degree, and magnesium sulfate the least. In fact no difference can be observed in the records between a suitably numbing dose of magnesium and no anesthetic at all. This degree of anesthesia however does not prevent an occasional violent kick from the animal's hind legs, which is liable to disturb the electrodes on the brain, and later experiments were conducted after recovery from ether, the spinal cord having been transected and the margins of wounds painted with novocaine. Since the most serious complication arises from poor blood supply to the cortex, and since stretching out the animal's hind legs engorges the abdominal pool with blood, after cord transection the animal's hind legs were placed under him, and 50 cc. of Tyrode's were injected intraperitoneally. The eye was removed and the optic nerve laid bare of muscles, but not dissected clean, as it was found that the optic nerve soon became non-irritable if its blood supply was cut off. The posterior cortical lobe of the opposite side was exposed and the dura removed.

A ring support 12 cm. in diameter clamped to the head-holder carried electrode holders that could be slid around to make any angle with the midline, and each holder carried an electrode wire sliding through a ball-and-socket joint, allowing a wide range of movement (fig. 1). Electrodes of silver and silver chloride were used, and from each holder a connection passed to each of two multiple-contact switches. To the control arm of

each switch was attached one of the leads to the cathode ray oscillograph-amplifier recording system, so that any electrode could be connected to grid or ground lead at will, or one of several electrodes on different cortical areas selected. For stimuli, condenser chargers were employed (Bishop and Heinbecker, 1930) a threshold response in a sensitive preparation being the current from a 0.01 m.f. condenser charged at from three to six volts.

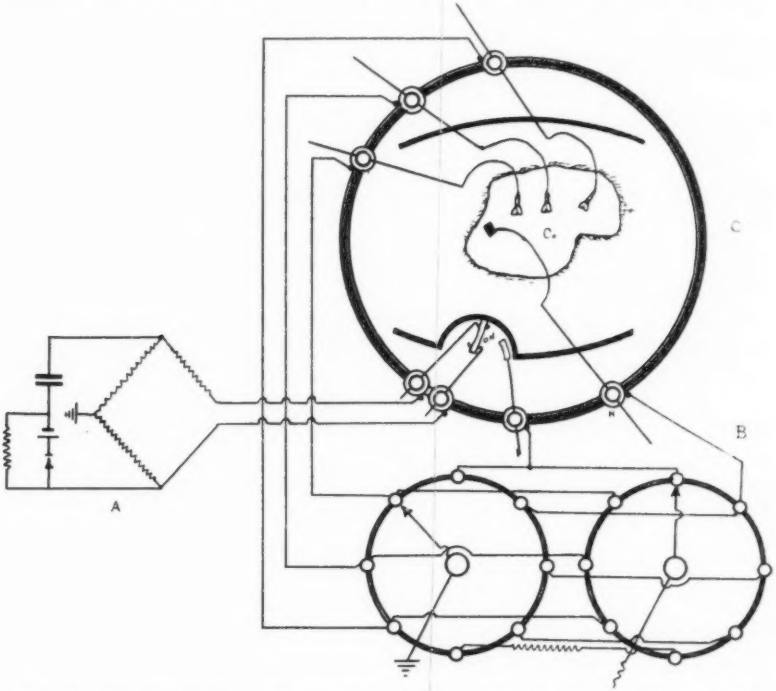

Fig. 1. The stimulating and recording arrangement. It consists of *A*, bridge stimulator which reduces shock artifact; *B*, the two rotor switches in parallel providing for use of any two leads; and *C*, the ring support holding the electrodes, each secured to it by adjustable ball-and-socket clamps similar to *H*. *Co.* is the optic cortex, and *ON*. the optic nerve.

Since this stimulating current was passed through a symmetrical bridge to reduce distortion in the record from the stimulus escape (fig. 1), the current actually passing through the nerve in one arm of this bridge was one-half of this value, and the threshold varied to considerably higher values depending on the cleanness of dissection, diameter of nerve, and probably on blood supply to the optic nerve fibers under the cathode.

Two methods of recording were employed. In the first (fig. 2), a rotary

circuit breaker timed at about one per second, had a series of contacts arranged as follows. One contact caused the oscillograph beam to pass too rapidly to be visible from right to left, and upon its break, the beam moved from left to right at a linear rate controlled by a condenser charged through a vacuum tube (Bishop, 1929). This gave a time coördinate, and with five lugs on the contact wheel, five lines were described on the face of the oscillograph during each cycle, three or four thousandths of a second being lost each time the beam returned from right to left (fig. 2). A second single contact at each cycle caused the beam to move abruptly upward

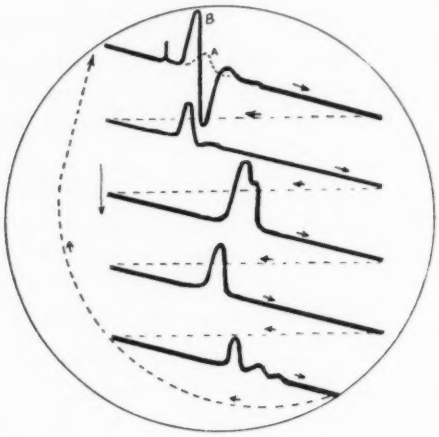

Fig. 2. Diagram of response seen on the face of the cathode ray oscillograph tube showing the five lines of activity occurring one after the other in time. Each solid line represents approximately one-fifth second as the return of the beam from right to left occupies only four or five sigma. The whole cycle immediately repeats as is suggested by the dotted line. Thus potentials appearing directly under each other in the succeeding lines are one-fifth second apart.

to the top of the oscillograph, the spot then moving slowly downward during the remainder of the cycle. Thus these five lines appeared successively one below the other at each cycle of the interrupter. A third lug was arranged to engage a toothed pawl, one tooth being advanced at each cycle, the wheel being set by depressing a foot switch. When the foot switch was down the beam was deflected far to the left, off the oscillograph screen, the tube being therefore dark. When the foot was lifted, the first tooth engaged at the next succeeding revolution of the interrupter caused the five lines to appear on the oscillograph, and a cut film placed in contact with the oscillograph during the previously dark period recorded the cortical response during one cycle. The second tooth being engaged, the oscillograph was again dark-

ened as before for one cycle, during which the film was removed without complication from a repetition of the picture; and at the next cycle the record was again allowed to appear on the oscillograph for visual observation. Three more contacts on the interruptor served to deliver one, two or three stimuli to the optic nerve at any desired intervals, and still another threw a calibrating voltage in series with the electrodes to the animal.

Now if the interruptor turned at a speed of once per second, and if, following a single stimulus to the optic nerve, the typical waves appeared in the cortical record at one-fifth second intervals, successive waves appeared one in each line, and each directly below its predecessor (fig. 2).

The other method of recording employed a second oscillograph set up in parallel with the first, fed by the same amplifier, but without the mechanism for producing a time coördinate. The oscillograph beam therefore moved only perpendicularly to follow the record. A 60 mm. wide film or bromide paper was then fed through rollers and guides which held it in contact with the oscillograph screen, producing the time movement. Time in seconds or fifths was marked on the record by making a contact in a circuit close to the amplifier input but not connected to it, which caused a succession of sharp breaks in the base line. The extended record could thus be watched on one oscillograph while being photographed on a moving film by the other. Records up to one meter per second were made on bromide paper.

Unless otherwise specified, a ground electrode serving as an indifferent lead was placed on the side of the head on injured tissue, and one or more point electrodes consisting of no. 30 silver wires were situated at the points on the cortex to be recorded from. Each wire electrode was protected by a broadened base made of celluloid to prevent its injuring the cortical surface, the wire ending flush with the flat surface of the celluloid. The stimulating circuit was also grounded through the bridge employed. Various tests of these leads indicated that the activity recorded was assignable to elements well within two millimeters of the point electrodes.

A sensitivity up to two meters per millivolt was available, but could not in general be employed profitably, because the finer details which it might have been used to detect would only appear superposed upon the large waves of the record, which at such a sensitivity pass beyond the limits of the oscillograph recording surface. Sixty to two-hundred millimeters per millivolt served for the average preparation, and at this sensitivity no serious disturbances from the animal or from stray fields enter into the record. In a room at 70°F., it was not found necessary to apply heat to the preparation.

Spontaneous activity and response to stimulation. The rabbit's cortex, like that of the dog (Bartley and Newman, 1930), is spontaneously active, so that whatever response to stimulation is obtained must be superimposed upon the considerable amount of activity that is already present. If the

response is small it may be hopelessly confused in the spontaneous picture, but if large and not too variable, it can be identified with certainty.

The amplitude and other aspects of the recorded *spontaneous* activity vary from place to place upon the cortex. Generally, if not always, in the passive animal, the greatest "spontaneous" activity, (that is, not due to a specific experimental stimulus) is found in the anterior half of the cortex. Inasmuch as no response from stimulating the optic nerve is obtained from within this area, relatively little exploration has been made there. The specific response to optic nerve stimulation is found only on the posterior half exclusive of the median portion bounded by what in the carnivora is the lateral sulcus, and where it is found, it varies somewhat from place to place. In general, within the optic cortex, the regions showing the greatest spontaneous activity give the lowest specific response to stimulation. A very low, but otherwise similar response may be obtained from the homolateral cortex.

If, in a passive animal, a brief but strong electrical shock is delivered directly to the optic nerve, one observes rising out of the record of the spontaneous activity of the contralateral cortex in the occipital region, a series of large waves easily recognized by their size, and by their stability of location upon repeating the stimulus (fig. 3). The approximate spacing of the major components of this series is so near to being one-fifth second that one can use this interval as a guide in looking for them. The first of these five waves follows the stimulus by a few sigma, and is often liable to be confused with the record of the shock itself. It is also, when maximal, always the largest of the series.

Careful analysis of this first element following threshold stimuli to the optic nerve shows that it is not simple, but consists of two responses superposed in the record, one small, and monophasic, appearing in response to the weakest stimulus, and a second, at slightly higher threshold, which is diphasic. As the stimulus is increased, the second component appears before the first has become maximal, and grows so rapidly that it is impossible to say how large a portion of the total is to be assigned to the monophasic wave. In a few preparations where the interval of the threshold for these two components was greater than usual, it was possible to establish the fact that even below threshold for the diphasic element, the first monophasic wave was followed, at about one-fifth second intervals, by at least three of the series of waves that became prominent with stronger stimuli. It is therefore inferred that there are two more or less independent responses of the cortex, one so far as we know single, that is, consisting of a simple diphasic wave following each stimulus by a short interval, the other complex, that is, consisting of at least five waves, generally monophasic in our records, and occupying more than a second after a single stimulus. The first phase of the diphasic element is almost always *positive*, that is, it

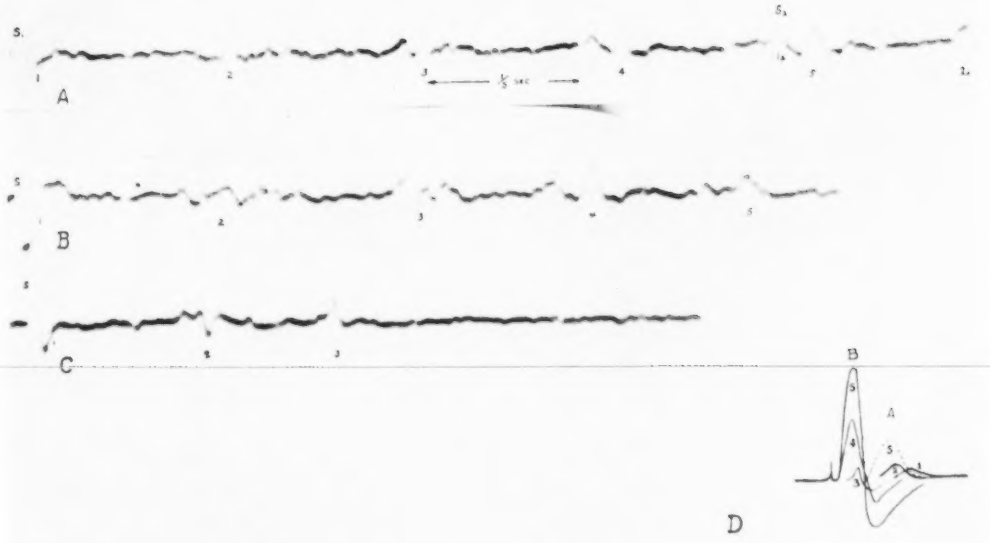

Fig. 3. Some typical responses. Time in one-fifth second is indicated by downward thrusts in the lines as is labelled in record *A*. *S*, *S1*, and *S2* show location of stimulus. The components of the series are numbered. Record *A* contains the complete series of five components as does also record *B*, while record *C* exhibits only three. In record *A*, a second stimulus is given before the completion of the first response series. *D* shows the shape of the first component at various stimulus intensity levels (see text). Records ⅔ size of originals.

records as a positivity at the surface of the cortex. The first of the series may be either positive or negative. A more detailed analysis of the meaning of the polarity we find in these records will be undertaken separately but for the present we wish to avoid giving the impression that we believe that the nervous elements responding become themselves *positive* with activity. Relative positivity at the surface means only relative negativity directed away from the surface, and quite plausible assumptions as to the polarity of the responding elements will account for most of these records on the conventional basis that nerve activity gives a negative potential sign.

In the passive rabbit, the longest "spontaneous"[2] waves are about one hundred sigma in total duration, with several other orders of waves present at the same time, the most prominent among which are those about eighteen sigma in duration. On the other hand, in the animal which is either actively tense or squirming, the original waves are complicated by others several times as high and only a few sigma in length. These are due both to vibrations of the electrodes in contact with the preparation, and to stray currents from muscle activity, and presumably are not assignable to the optic cortex.

It appears that light anesthesia does not noticeably alter the records of spontaneous activity just described. We have not yet been able to demonstrate that there is any feature of the pattern disturbed by the amounts of anesthetic that we have used to immobilize our animals. Deep anesthesia does, of course, reduce the amplitude of the potentials to extinction, with an intermediate stage where the record becomes simpler and more repetitive than normal.

The highest elevations of the spontaneous record are not over one-fifth millivolt, although occasionally in a rabbit which is restless, larger single potentials not due to optic nerve stimulation seem to be assignable to activity of the optic cortex. On a base line whose average deviations are less than one-tenth millivolt, the responses to stimulation in a good preparation rise from one-half to one millivolt, and the first diphasic wave may show each phase larger than this. The durations of the individual potentials of the responses are never less than about twenty sigma, when single and very sharp, and in place of such a single wave, there may appear a group of several lower waves of similar duration. It appears that the spontaneous activity could be interpreted as the algebraic sum of a large number of simple rhythms, more or less out of phase, each unit being of the order of duration of one of the units of the record following stimulation of the nerve.

[2] By "spontaneous," we mean not initiated by a specific stimulus to the optic nerve. Whether other afferent stimuli cause such activity we do not know, but we have some evidence for rhythmic activity that may arise in the central nervous system itself.

No waves have been detected with durations of the order of those of nerve fiber action potentials in peripheral nerves, and other considerations lead us to believe that nerve fiber potentials do not appear in our records, but that these potentials are assignable to cell bodies or to specialized nerve endings associated with them. With a constant repeated stimulus, records from different parts of the optic cortex vary in amplitude and may vary somewhat in time, but there is no indication that the impulses we record reach normally certain parts of the cortex directly and then propagate laterally across it. Rather a complete record is assignable to elements responding similarly at each point of the cortex. Near the edge of the active area the record is lower, and the later waves drop out, leaving only two or three recognizable; and in a marginal area the first diphasic potential can be elicited where the series of monophasic potentials, if not actually absent, is perhaps too low to be visible above the spontaneous activity of the cortex.

Cortical responses to single stimuli increase in amplitude with increase in strength of stimulus to the optic nerve, until two or three times the threshold value has been reached. This is obviously an effect of stimulating more nerve fibers. Furthermore, the threshold and maximal stimuli appear to be the same for all parts of the optic cortex; for instance, stimulation of only the most irritable fibers of the optic nerve causes a response everywhere in the cortex, but this response is everywhere a weak one. On the other hand, when a single stimulus calls forth a just *maximal* response, not all the fibers of the optic nerve have been stimulated. This is shown by the effect of two stimuli a second or two apart. The second series of responses is not maximal unless the second stimulus is considerably *above* the maximal value for a first response. Apparently the relationship between a given optic nerve fiber and a group of nervous elements responding in the cortex is not a simple one to one relationship. The greater the number of fibers stimulated, the greater may be the response of each group of elements in the cortex.

This becomes more evident from the results of repetitive stimulation. A weak stimulus repeated at any interval does not give constant responses. A stimulus which gives an occasional maximal response when repeated, may also give any amount less than this. No stimulus however strong will give a second response as large as the first with an interval of less than three seconds between them. A just maximal stimulus is therefore taken as one that, when repeated at three second intervals, gives an occasional maximal response; a threshold stimulus is likewise one that gives an occasional just visible response. A stimulus considerably stronger than the just maximal one will usually give a maximal response to each repetition at three second intervals or more. With more frequent stimulation than this is no simple statement can deal adequately, and the events will be analysed elsewhere.

In what follows here, responses to single or slowly repeated stimuli are concerned.

Under the most constant conditions we can obtain, the responses of the cortex vary from each other in details. The existing variation depends, among other things, upon the strength of stimulus used, and the place upon the cortex recorded from. Table 1 gives some representative times of occurrence of three of the components (the second, third and fourth) and the intervals between them at different points of the cortex; times are measured from the stimulus.

The series itself varies in length due to the number of components in it and to the spacing of the latter. Under some experimental conditions,

TABLE 1

Times of responses of cortex to stimulation of optic nerve

Column *2*, time after the stimulus of second response. *3*, time of third response. *2–3*, interval between second and third, etc.

2	3	2-3	4	3-4	CORTICAL POINT	RABBIT 19
230	415	185	612	197	I	All maximal stimuli
220	394	174	581	187	I	
210	384	174	—	—	I	
197	394	197	606	212	II	
164	384	220	592	218	III	
230	399	169	—	—	I	
223	427	184	—	—	I	
						RABBIT 20
233	470	237	703	233	I	
233	470	237	695	225	I	22 v. just over threshold
185	392	207	629	237	II	
198	383	185	629	246	II	

namely, when certain drugs are used on the cortex, only the first component remains, though the usual normal response consists of five components. At times the response has been observed to last well over one second, each interval being longer than usual. A second series of responses can be started before the first has subsided without serious interference.

The individual components consist, at times, of single large relatively simple waves; in other preparations they are represented by a group of lesser ones. If this group consists of but two waves, the latter may be located symmetrically with reference to the place a single wave would be expected to appear, and they may vary from being partly fused to being separated by a number of sigma. When a component is represented by four or five or even more waves they are much smaller than when fewer in number.

Not all components in a given series are similarly broken up into smaller waves. Some may appear entirely simple while others show varying degrees of complication. The members of any series are generally widely enough separated in time so that they can be identified even when so broken up. Consequently, one does not find hopeless confusion in a picture which is by no means simple.

Within a response of whatever length it happens to be, it is quite common to find one or more components lacking or vanishingly small at an amplification where the rest are large, there being no certain ones which, as far as we know, are more likely than others to be absent, except that the last two are often small. The first and second are frequently followed by waves which seem to be interpolated extra elements, rather than parts of the typical series. The chief variations in this aspect of the response arise as one goes from one animal to another or from point to point on the cortex. With any one location upon a single animal there is great constancy in this respect during an experiment lasting for an hour.

When a response is characterized by a series of simple members, they may be all monophasic and of the same potential sign, but even in this variety presents itself, and individual members may be of opposite sign. Monophasic components are usually observed except for the first diphasic wave, but both diphasic and triphasic waves occur sometimes even within a single response series along with monophasic potentials. Two of the salient conditions for variability here also seem to be the animal used and the point of the cortex recorded from.

It has been stated that the response varies with repetitive stimulation even if at a rate of no more than once per second. However, if the stimulus is maximal or above for the first response, the characteristic alteration to be observed is the diminution of the amplitude from shock to shock, when after about three or four are delivered the height of the response comes to a nearly constant level, and at times may remain so, indefinitely. On the other hand, if the stimulus is not much above threshold, at one per second, after only a few occurrences the response may entirely disappear. Then again, only alternate stimuli may be effective. Another type of variation is the simplification of the picture after two or three stimulations due to a change in the response itself, and possibly also to a change in the spontaneous activity. A third kind of change occurs as stimulation is continued, namely, that one or more of the components in the series appear progressively earlier and earlier until perhaps four shocks have been given. For example, in one animal, the second component which consisted of a large rather simple monophasic wave came much later than a fifth of a second after the first, but shifted completely up into place by the time that it had made its fourth appearance.

Most attention has been given to the first component of the series. In it

two elements have been identified, which though they distinctly vary, are more nearly constant in form than the other components. Under some conditions they appear at threshold definitely separate from each other, though at other times the one is superimposed upon the other. They may be manipulated by at least two means: first, by varying the stimulus strength, and second, by the use of drugs upon the cortex. It has been regularly found that the threshold of the one which is monophasic, designated in what follows as *A*, is lower than that of the diphasic wave, *B*. They may differ by as much as the ratio of one to three, usually less. Sometimes the usual series of five waves appears before the threshold for *B* is reached; *A* is then smaller than the following waves. It is probably never maximal below the threshold for *B*, but is always small compared to the latter. With additional stimulus strength, *B* rises to many times the original value of *A*. Certain examples are cited to show the variation in appearance of these waves.

I. In some instances *A* is first observed as a low prolonged wave as shown in figure *3D*. With further increase in strength of stimulus, a second wave of about the same size or slightly larger suddenly appears just in front of *A*, which itself moves earlier as the stimulus is increased. *B* now becomes much larger with added increments in stimulus intensity, until it obscures the form of *A*.

II. At other times *A* first appears as a small wave which suddenly changes shape when the stimulus is increased. This transformation is shown in figure *3D*. Upon closely watching the phenomenon, one can see that a second wave which is diphasic appears just in time to be superimposed upon *A*. Increasing the stimulus in this case increases the amplitude of what is observed as the diphasic constituent, *B*, with the monophasic wave obscured from the start.

III. In this case, before any pronounced wave is observed, there exists a very long though slight elevation of the base line in the region where the waves are expected to emerge. With a stimulus increase, this elevation is replaced by one and then two slight humps completely separated from each other. *A* is later shown to be next to the stimulus. When the stimulus is further increased, the late hump becomes relatively much larger than the early one and at the same time fuses with the former, becoming visibly diphasic.

IV. Here we find the threshold response, or *A*, a single slight hump in the base line. *B* appears at a higher threshold and later, apparently being monophasic and directed oppositely to the usual case. With stronger stimuli the larger record becomes definitely diphasic, but the first phase remains much smaller than the second. That is, a first phase too low to be certainly visible at the start, increases in amplitude in its proper position ahead of the second.

V. We may incidentally mention here that there is a stage in the poisoning of the cortex by strychnine directly under or close to the exploring electrode, when *A* and *B* may be widely separated in time, with *B* varying in its time of appearance from almost complete fusion with *A* to a separation of about one hundred sigma or more.

DISCUSSION. While the difference in threshold for these two components is sometimes slight, we think the evidence is fairly clear that the potential designated as *A* is the first element of the series of five similar waves, while *B* is distinct from this series. First, *B* is always diphasic, all the rest are usually monophasic or but slightly diphasic. Second, when the thresholds do differ significantly, the whole monophasic series may appear below the threshold for the diphasic element. Third, strychnine abolishes the series of five monophasic waves, but increases the amplitude of the diphasic response. The question then arises, are there two parallel pathways from retina to cortex, that is, do different fibers in the optic nerve initiate two different responses in the cortex; or is there a separation of pathways higher up, say, in the thalamus. In spite of a difference in threshold for the two effects, the latter is probably the true state of affairs. If a shock above threshold for both effects is repeated at one second intervals, both responses fluctuate together; that is, when this diphasic wave is high, the rest are also high, and vice versa. It cannot be supposed that the optic nerve fibers differ so widely from the fibers of peripheral nerves as not to respond equally at one second intervals to a uniformly repeated stimulus. The variation in response must be determined at some cell station along the pathway. The fact that this variation occurs equally in both types of responses indicates that they may have a part of their paths in common. Not only this, but the facts that when more fibers are stimulated, the responses occur earlier, and that a stimulus which gives an *occasional* maximal response must be increased materially, (i.e., more fibers must be stimulated) to give a maximal response every time, indicate that the excitation of one fiber of the optic nerve may increase the response caused by the excitation of other fibers; that is, the greater the number of fibers stimulated, the greater the effect on each element responding. This is not necessarily contrary to the idea, supported by good evidence, that points in the retina are localized at points in the cortex, a situation which must require specific fiber pathways to connect such points. It only requires the further assumption that the activation of one such pathway affects the ease with which parallel pathways may be activated, and this is quite the rule in the central nervous system. In particular, the evidence suggests that it may require the activation of more than one fiber to cause any response at all, which would only be another instance of the summation so common in nervous activity. The degree of summation required to bring about the two responses we observe would then be different, and might differ in different prepara-

tions, corresponding both to the variations in thresholds for the two responses and also to the variations in the relative times after the stimulus at which they begin.

As to what these two responses might mean in terms of vision we cannot hazard a guess at present. It is highly improbable that it requires a second of time for a rabbit to see a dog; it might take that much time for him to complete his whole visual activity with respect to what he "saw" more immediately. A more serious obstacle is offered by the fact that the cortex does not respond continuously, but is refractory or depressed for some time after a single stimulus; but the rabbit presumably gazes upon his environment unembarrassed by the periods of cortical blindness suggested by such findings. It is possible that our procedure of stimulating all the fibers of the optic nerve at once, adopted as a means of simplifying the record of the response, has rendered it too simple to be interpreted in terms of vision, although still too complex to be analysed in terms of nervous pathways. A further possibility is that the mode of action of the cortex is different enough from that of lower centers and of peripheral nerves, so that the concepts of neurological functions founded on a study of lower levels do not apply to cortical activity. At any rate, any concrete interpretation offered at this stage could only bias future investigation, and further conclusions are therefore postponed.

SUMMARY

Herein, we have described the cortical response to stimulation of the optic nerve which can be observed rising above the continuous spontaneous activity recorded from the cortex.

It consists typically of a series of five major components occurring at approximately one-fifth second intervals.

A number of variations in the constituents of the series is pointed out, among which are the complexity of the components, the frequent omission of one or more of them, the occasional reversal of potential sign of some of them.

An analysis of the first component shows it to be composed of two waves, one monophasic with a low threshold, and the other one much larger and diphasic with a higher threshold, superimposed in the usual record.

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FACTORS DETERMINING THE FORM OF THE ELECTRICAL RESPONSE FROM THE OPTIC CORTEX OF THE RABBIT

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Electrical stimulation of the optic nerve of the rabbit (Bartley and Bishop, 1933) results in specific electrical responses of the optic cortex superimposed upon a considerable degree of cortical activity continuously present. In attempting to interpret these cortical action potentials in the same general manner as the potentials from peripheral nerve trunks, we have met with certain complications both mechanical and functional with which it is the purpose of this paper to deal.

In analysing the electrical records obtained from the activity of peripheral nerve trunks, conditions may be much simplified by removing the nerves in question from the body, but this is not feasible in recording the activity of the cerebral cortex of higher animals, owing to the much greater dependence of the activity of the nerve cells upon blood supply. The pathway over which the impulses travel, essentially linear in nerve fibers, maybe extremely complicated in such a system as the cerebral cortex. Thus both the selection of suitable recording arrangements and the interpretation of the results when they are obtained are made difficult and precarious. In this system, there are several types of nervous structure to take into consideration, cell bodies, various arrangements of fibers, and their synapses, whose rôles in the total activity are presumably very different. In connection with complications to be expected from the diversity of elements, there are shunting effects of tissue masses to reckon with, such that the amplitude observed in a record of their activity depends not only on the amount of intrinsic activity, but on the position of the reacting element. Further, disturbing phenomena foreign to the specific nervous tissues themselves enter into the records in subtle ways and may not be discovered until one has accumulated a great deal of experience in searching for them. These influences may be classed as mechanical artifacts and electrical "pick-ups" from disturbances in the animal or in the space surrounding it.

A number of tests were made to pick these out if they existed, and make

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sure that the patterns exhibited upon our records represented the activity of the cortex itself. The first was the insertion of a 100,000 ohm resistance in the input circuit close to the animal; at the same time, the animal was connected to the grid lead of the amplifying system to act as an antenna. If any significant portion of the pattern of the response were due to the animal acting as a "pick-up," this arrangement would supply favorable conditions for bringing this to light. However, the base line was quiet with this arrangement, indicating that with the type and placement of the leading-off electrodes employed, we were not inadvertently obtaining spurious effects.

Two types of mechanical disturbance might enter in to produce artifacts in the records: vibrations of the operating table upon which the animal rests, or voluntary movements of the animal itself displacing the electrodes.

The incidental occurrence of the first was ruled out by test, and the occurrence of the second was found to be rare. With tensing and movement of the animal, very large excursions did take place in the records, however. These, by their difference from the continuous activity observed with the animal in the passive state, could always be distinguished and the records containing them discarded. Counts of respiration and pulse were made in connection with the major excursions in the patterns. The results showed that there was no observable correlation. The electrodes, both placed upon the muscle tissue of the head, failed to record any kind of activity which compared to the continuous flow of waves when recording from the cortex.

Certain other phenomena encountered in our experiments will also indicate the validity of these records. For example, the diminution of the potentials by deep ether anesthesia and their obliteration at the death of the animal, and their dependence upon cortical blood supply show the activity is from the brain, and more localized tests show it to be of cortical origin. Both the genuineness of the records and the direction their interpretation must take will be considered in connection with experiments along the following lines:

1. The responses from direct stimulation of the cerebral cortex obtained in a few cases under special conditions.

2. The effects of cutting off the cortical blood supply and the isolation of parts of the cortex by incision as evidence of the cortical locus of our recorded activity, both spontaneous and from stimulation.

3. The effects of general anesthesia and its comparison with the unanesthetized state.

4. The question whether the potentials developed are those primarily of cells or of fibers.

5. The question whether the cortical responses to stimulation of the

optic nerve are the repetitive activity of a single group of elements or the successive discharges of diverse groups.

6. The significance of the appearance of both monophasic and diphasic waves.

1. *Direct stimulation:* In several cases, an attempt was made to stimulate the rabbit's cortex directly by placing four electrodes in a line, two of which were stimulators, the cathode being adjacent to the leads. So far, however, failure to demonstrate a specific response has attended most attempts of this kind. In two cases in which an area of cortical tissue was selected that was supplied by one artery and one vein, we were successful. An incision was made around three sides of this in such a way that it could be lifted upon a small metal platform which served as a grounded lead, while leaving the blood supply functionally intact. In each of the animals, the cortical tongue showed "spontaneous" activity which was traced by way of the intact connection with the rest of the brain. In one case, the activity was relatively simple and consisted of a series of waves having a frequency of about three per second. In order that these might appear as standing waves on the face of the oscillograph tube, the revolution of the interrupter was made nearly synchronous with them, although slightly slower, so that successive responses progressed very slowly across the face of the tube. When a stimulus was sent into the tissue at each revolution of the interrupter, it fell later and later in the succession of waves. A stimulus in the positive phase was ineffective, but as the stimulus fell later in the cycle a progressively larger response was elicited, each consisting of a wave slightly shorter than the spontaneous wave. This tended to inhibit the following positive spontaneous wave, the more, the smaller the interval by which it preceded the latter, so that in some cases there was a compensatory pause without permanently interrupting the rhythm which was imposed from without the incised region. Tying off the artery stopped all activity almost at once.

The interpretation of the failure under ordinary conditions to elicit an observable response from direct stimulation of the cortex, is that no point-like stimulation is sufficient to set simultaneously into action a sufficient number of elements for the production of a potential which would be observable above the continuous spontaneous activity found there. Thus for all practical purposes, the effect is swallowed up. If this be the case, then it could reasonably occur that success would now and then attend the attempts to stimulate such an isolated tongue of tissue. In it a simple pattern sometimes occurred, such that there was at any one moment a greater proportion of the cells at a given stage of activity or recovery. With synchronization of this kind the spread of current from punctiform stimulation might well be effectual, whereas with large numbers of cells at all stages in recovery nothing identifiable would appear in the records. Success would

obviously depend also, in the above experiment, on the presence of a functional nervous pathway via the uncut tissue under the entrance of the blood supply.

2. *Cortical isolation and removal of blood supply:* Areas were circumscribed in a number of animals, step by step, by means of incisions, to test whether a small portion of the cortex could be wholly isolated from its lateral connections and still exhibit some sort of activity. Precision in making such isolations is scarcely possible, for if one fails to cut deep enough, some lateral connections may remain to account for the activity obtained. On the other hand, if the incisions are deep, projection fibers are involved in the damage, for such fibers do not reach the cortex by a truly perpendicular approach to it. In any case one will damage the blood supply, so that little time for observation is available on account of the alteration and diminution of the activity in the isolated area.

An area may be almost encircled by incisions and yet show little change in the activity first noted there. This is true when not a great deal of time has been allowed to elapse. At times, when an additional cut is made around the area mentioned to further circumscribe it, the activity disappears by the time a record can be obtained. On the other hand, if an area is partially circumscribed, the activity may persist for five to ten minutes, or indefinitely, the time probably depending upon the degree of interference with the blood supply. Sometimes the first small cut would stop all activity; presumably the nervous pathway over which the impulses reached the area was broken.

It was found with the use of long transverse cuts that, in some cases, the response to stimulation of the optic nerve anterior to the cut was abolished while that on the posterior side of the cut was not. Such a transverse cut might be so placed as to divide the cortical area responsive to optic nerve stimulation into a posterior and anterior section. The area in front of the cut, when the records were abolished, must have been deprived of optic projection fibers. If the records we obtain normally with leads on the cortex, from stimulation of the optic nerve, included records of the activity of the optic radiation fibers rather than of specifically cortical elements, we should still expect to obtain records of activity anterior to the above mentioned cut. That is, with one lead on the cortex even anterior to the cut, and the other on the side of the head as usual, the optic radiation still lies between the electrodes almost as effectively as if the cortical electrode were just behind the cut; but this would not hold for the registration of the activity of elements at the cortical surface. No response is obtained anteriorly, even at a millimeter from the cut, indicating that the locus of activity from which we record is limited to a very small area about the electrode on the cortical surface.

The observed *gradual* disappearance of the spontaneous potentials

after cutting the cortex would seem to be due to the dependence of the responding elements upon blood supply and further, these elements must be located in the cortex, for it was only the cortex that was deprived of blood supply. Since nerve fibers do not exhibit that dependence, in experiments on peripheral nerves, it may be concluded we are here dealing with the activity of nerve cells or their synapses rather than fibers.

The dependence of cortical activity upon blood supply was also demonstrated incidentally a few times when the cortex became pale, either before or during recording as a result of poor heart action. In each case, the recorded cortical potentials became very weak and the animal became unusable for our ordinary experiments. In some cases the cortex was periodically pale and then somewhat better supplied with blood. It was found that these changes were accompanied by corresponding changes in the amplitude of the cortical activity. Direct tying off of a superficial artery without injury to the underlying tissue caused a gradual decrease of activity recorded from the region supplied by it in which case activity ceased in from three to five minutes.

3. *Effects of general anesthesia:* It is common doctrine that general anesthetics have a preferential effect upon the cortex inasmuch as they abolish consciousness before the activity of the lower centers. The ideal anesthetic for our purposes would reverse this order. Three different anesthetics, sodium amytal, ether, and magnesium sulphate were tried. Ether had many obvious disadvantages, among which were its disagreeableness in a closed dark room, and the difficulty of maintaining a constant level of anesthesia. Consequently sodium amytal was tried, and discarded because of the likelihood of administering an overdose. The use of magnesium was certainly not without its hazards, chief among which proved to be the frequent occurrence of heart block, but of the three it proved the best for our purposes. Its action could be controlled to a degree by the injection of calcium chloride; there was no need of constant administration, and with the amounts found necessary to quiet the animal, there was the minimum of cortical depression.

In a number of cases, using both dogs and rabbits, we prepared the animals under ether and then took our first records while they remained sufficiently under its influence to be quiescent. In this state, the amplitude of the spontaneous continual activity of the cortex that has been described elsewhere was nearly normal. The animals were then allowed to recover from the influence of the ether to the extent of regaining the ability to move about and to respond to weak external stimuli. Records now taken for comparison were found to be the same in character as those previously obtained, except that the potentials were higher in amplitude. Following this the animals were gradually put deeper and deeper into anesthesia, while the potentials were watched. These did not become greatly reduced

until the anesthesia became deeper than we found necessary for simple immobilization of the animal. At a deeper stage, for a moment, the wave patterns became relatively simple. Very soon, however, their simplicity disappeared and was replaced by a much lower amplitude of wave, again complicated in pattern. That this simplified pattern was not a fortuitous occurrence, was shown by its recurrence in two other animals. Finally by the time that the last reflexes were being abolished and breathing and heartbeat were about to cease, the potentials had become so small as to be counted negligible. The records obtained from animals under general anesthesia were compared with those gained from animals which were given local treatment with novocaine. This comparison also showed that the depth of anesthesia used for immobilizing our animals did not materially affect our records.

The facts show then that the gross record of cortical activity is essentially the same in the ordinary light levels of anesthesia we used as it is in the waking state, as judged by action potentials, with the exception that the potentials are a little lower. Cortical effects of anesthesia might be due to blocking of impulses from the periphery, rather than to depression of cortical activity as such, quite as motor ability disappears before consciousness of the attempt to move. The question, however, might arise as to whether the electrical activity we are measuring is a significant index of the activity of the cortex in and out of anesthesia. We think it is for the following reasons. In the first place, we have by various checks made sure we are actually recording the activity of the cortex itself; some of these have been referred to above. Secondly, the activity we measure varies under changing experimental conditions and spontaneously in a manner that might be expected of cortical activity under the same conditions. The type of picture varies with muscular tenseness, with the ampleness of blood supply, with the action of various drugs, from individual to individual, and from time to time in the same individual. That is, in general, action potentials are affected by agents that affect other manifestations of cortical activity, and in the same direction. The fact that the amplitude of such potentials seems to go more nearly parallel to reflex activity than to the more complex behavior that is usually thought of as conscious, may mean that we are not recording the potential *details* which would give us information of the more highly structured activities. Or, it may mean, as suggested above, that the anesthetic *isolates* the cortex before it depresses its spontaneous activity. It is not impossible that the differential conditions for "consciousness" as opposed to the "unconsciousness" of anesthesia do not lie in the cortex primarily. While a degree of anesthesia that might correspond to a loss of consciousness does not cause any great alteration in amplitude or form of the experimental potential, the cortical record is so complex that alteration in certain details would not be detected. An interesting question is

raised as to what diminished amplitude of potential might mean in terms of cortical function anyway, whether it involves fewer elements responding, or lower response in each element.

4. *Cells or fibers:* Both cells and fibers, of course, take part in cortical activity, but there is the possibility that difference in size of the potentials developed by the two might make one of them insignificant in its effect in the records. Our evidence points to the activity of cells as playing the dominant rôle in the records. Under cells, we would include synapses and specialized endings, for between these we cannot differentiate by potential records. The slow disappearance of the potentials after the area of the cortex recorded from was circumscribed by incision seemed to indicate that the activity died out at least partly because of the lack of blood supply to elements otherwise intact. Cells and not fibers would behave this way. If the cutting stopped the activity by severing neural connections, the activity should have ceased immediately, and not all incisions took effect at once.

The spontaneous activity of the rabbit's cortex exhibits potential variations ranging all the way from 15 to 100 sigma in duration, the amplitude of the larger waves being as much as $\frac{1}{2}$ mv. Responses to stimulation of the optic nerve may be several times higher. No oscillations emerge that are as short in duration as nerve fiber action potentials. Furthermore, these potentials are suppressed by concentrations of anesthetics which do not affect nerve fibers; although if synapses were blocked, the axons of succeeding cells would cease to respond, and this evidence is perhaps not differential.

Further evidence for cellular activity was obtained in an experiment to determine what the record of a bundle of fibers would look like when the fibers lay on or underneath the surface of the brain. A rabbit vagus nerve $\frac{1}{2}$ mm. in diameter was laid across the cortex of the animal, then in a slit with its surface just flush with the cortex, then buried 1 mm. deep, and finally it was threaded through the ventricle of the brain. In each position, the nerve was stimulated at one end and action potentials led off by way of electrodes on the cortex as close as possible to the nerve. These potentials were 6 per cent, 5 per cent, 3 per cent, and 1 per cent, respectively of those developed by the same nerve in air. This difference is due to the shunting effect of the tissue mass. On this basis, it can be computed that if all of the fibers in the cortex within 1 mm. of the lead electrode were active at once, their summed potentials would be shunted by the rest of the brain to about 3 per cent of their value when insulated, and the record of fibers at greater distances than 1 mm. would soon reach the vanishing point. To produce the large spontaneous cortical waves which are up to 100 sigma long, a prolonged succession of discharges would be required if the waves were due to a summation of fiber potentials. In order that a sufficient number should discharge at any instant to account for the height of the observed potential,

all the more fibers would be necessary. Calculating from these facts, one would infer that to produce records similar to those regularly obtained from the rabbit's cortex, would necessitate fibers whose activity equals that of over one thousand vagus nerves to be gathered within perhaps 2 mm. of the leading-off electrode. But there is not room in that small range for so many fibers. Or, for example, if each of these hypothetical elements developed a wave of potential similar to a 15 mu myelinated fiber from the bull frog, 250,000 such fibers would be required to build up one cortical wave. The cross section of this bundle would be about 44 sq. mm., if tightly packed. Close arrangement is not the case, and much of the space is occupied by nerve cells and neuroglia, so that with the rabbit's cortex only 3 mm. thick, much greater potentials must be developed by each individual element than would be allowable for nerve fibers. It is, therefore, inferred that cells or specialized parts of them rather than fibers must produce these large potentials and, in addition, it is safe to assume that cell potentials would last longer than fiber potentials. This latter assumption would call for a lesser number of elements than if the discharge were rapid as is the case with nerve fibers. That cells may have an activity (whether a potential or not) lasting longer than nerve fiber action potentials is obvious from such phenomena as after-discharge; and Adrian (1931) has recorded such long lasting potentials which he assigns to ganglion cells.

5. *Responses of successive groups vs. repetitive responses of a single group:* The prolonged response of the cortex to each stimulus delivered to the optic nerve, described previously, shows certain features which lead one to believe that its several components are the successive responses of several groups of cells rather than simply repetitive responses of a single group. The first potential of this series can be shown to be double, (Bartley and Bishop, 1933) and the difference in form of its two component waves indicates that at least two groups of cells are responding. The question is whether the succeeding four responses can be assigned to one of these groups of cells.

In the first place, the major waves that compose the total response are unevenly spaced. Although we have stated that they commonly appear at one-fifth second intervals, the margin of fluctuation is considerably more than would be expected were the spacing that of repetitive activity of a single group of cells, discharging similarly each time. The objection might here be raised that no group of cells is, on statistical grounds, likely to behave twice very nearly alike. At first this seems to be a valid obstacle in the way of assuming that one can ever find out whether the activity we observe is that of a single set of elements or the activity of diverse groups. But on the other hand the fact that strychnine applied to the cortex results in a prolonged series of responses whose shape and size are similar to within

a fraction too small to consider, shows the possibility of exactly repeated activity. There can be no doubt that in strychnine poisoning there is repetitive action of one specific group of elements, and in that activity we find great constancy. Irregularity of the spacing then in the normal record, seems to be evidence for the supposition that the several waves represent the discharge of diverse groups of cells.

The amplitudes of these component waves are dissimilar and follow no simple rule as to size. The last two waves of the series are quite often small and sometimes absent; but they may be present when a component ahead of them does not appear, or is too small to be recognized. These differences in size are far too great to be due to simple statistical variation. The occasional dropping out of members in the response series further contributes to the belief that we are dealing with more than one group of cells. The gap formed by the failure of a component to appear in its turn shows at that moment either a cessation of activity of some group of cells or else such chaos that the potentials of the individual cells cancel in the record. The latter can apparently take place in the strychninized cortex. (Bartley, 1933.) If such a thing occurs in the normal cortex, the disappearance of a member in the series is a special case of the complications we have just discussed, and the return of the successive responses to simplicity is against the possibility that the same cells are involved. But if, on the other hand, true cessation of activity of certain cells occurs, we then lack the reason for the remainder of the components appearing at all in the series, if the whole series represents the repetitive activity of simply one group of cells.

Another feature of the response to stimulation is the occasional reversal of a potential, and here no simple rule has yet been discovered for such an occurrence. Many times the whole series is similar in sign, at others two or three successive waves are alike, while occasionally the reversal is alternate in simple fashion. If these waves resulted from the repetitive discharge of a single group of cells, these reversals within a single series would be hard to explain, for the leads remain at fixed points. This argument is weakened by the circumstance that we cannot understand these reversals on any basis.

As was pointed out in the original description of these waves, the components do not always arise as simple large waves. One might be represented by a pair of waves, or even a closely packed group of smaller waves. When groupings appeared, they were sometimes quite regular, while at other times complicated fusions supplanted this regularity. Whatever their nature, they seemed to represent a breaking up of the large wave that at other times was seen in their place. The important point here, however, is the fact that when complications did appear in the response series, all of the components did not participate similarly in them, for some waves remained simple, while others partook of varying degrees of complexity.

These waves vary in form as well as in number; monophasic, diphasic, and triphasic waves may appear in the same series of responses.

Yet another phenomenon opposes the supposition that all activity is that of one cell group. We should expect that if a second stimulus were delivered to the optic nerve before the cortical response to the first was completed, the two should sum in some way, and that two separate responses should not be elicited. But on the contrary, the latter can occur; the second response then begins before the first has ended and the two sets of waves keep their time relations in a seemingly independent manner and can be readily distinguished in the record. This suggests that the last cells active in the first response are not the same ones that are active in the beginning of the second.

In spite of all these considerations another suggestive possibility exists. We might assume that at some subcortical level, a group of cells whose potentials do not show in our records gives repetitive responses to single stimuli to the optic nerve. Each such response might then serve as a stimulus to the cortical elements whose potentials make up the recorded response to stimulation. Now if these elements are also the ones whose "spontaneous" activity (i.e., not resulting from our specific stimulus) gives the continuously varying record that we obtain without stimulating, we might conclude that the character of each specific response in the series of five depended upon the state of these cortical elements at the moment when the specific impulse from below reached them. The cortical elements are rather obviously periodic in activity even when not experimentally stimulated via the optic nerve. Varying phase relationships between the repetitive impulses from below following a stimulus and the rhythmic activity of the cortical elements themselves, might account for many of the variations in the records of the ultimate response to the stimulus. Experimental evidence on rhythmicity of response presented separately (Bishop, 1933) may support such considerations. This argument leaves at least two groups of cells responding to give two types of potentials, but leaves the question of what relation the various layers of cells in the cortex bear to our records unanswered for the present.

6. *Potential sign of the record and polarity of structure:* Of the two types of responses obtained, one is uniformly diphasic, its first phase connoting positivity of the cortical surface with activity; the other is a series of five responses all typically monophasic, again with the cortical surface positive, but showing frequent variations and reversals as indicated above. Since all cell activity so far as is known results in negativity at the active region, we infer that such records indicate the polarity of the responding elements; that these are in general directed perpendicularly to the cortical surface, and that the impulse in general passes over them from below outward toward the surface of the cortex. Positivity of the surface of the cortex

would then indicate negativity at the interior pole of each element, and vice versa. In the case of monophasic potentials, one would have to infer that an impulse either failed to sweep completely across an element, that is, that only the lower pole of the element developed a potential; or else that such an impulse having activated such a structure at the lower pole then propagated *laterally*, and that propagation in different directions then would cancel out in the record. No obvious explanation can be offered for *reversal* of polarity, unless it involves antidromic conduction, which we hesitate to suggest as an explanation of the normal record.

The leads we have employed, one point electrode on the cortex close to the responding element, and one indifferent at a distance, might be expected not to give a sharp diphasic record under any circumstance, and in simple muscle or nerve conduction, the phase representing negativity toward the indifferent lead is in fact suppressed (Bishop and Gilson, 1929). The record in such a case is monophasic or somewhat triphasic. The fact that one of the potentials recorded from the cortex is sharply diphasic with two phases almost exact mirror images of each other must indicate further the position and polarity of the elements involved. It correlates with the fact that cells of the cortex, although oriented perpendicularly, are arranged in a layer horizontal to that surface, and thus might act on the two electrodes as if they comprised a membrane interposed between them (fig. 1). If one side of this membrane develops a potential with respect to the adjacent tissue, it will not matter as far as the form of the potential is concerned whether the electrode is applied immediately to the membrane, or at a distance, to the conducting tissue in contact with it. As a result of the low resistance of this tissue mass, the potential at a distance will tend to be an average of that of all points on the membrane. Thus in this respect neither electrode can actually be an indifferent one, and the phase indicating negativity toward the distant lead will tend to record an average effect of all the elements responding in this layer; but the phase recorded as a negativity toward the lead applied closely to the elements responding will measure the activity primarily of the elements directly under the lead. That the two phases of the diphasic response are so nearly equal indicates that all elements of the "membrane" of cells respond at approximately

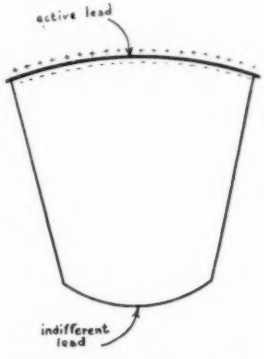

Fig. 1. Diagram of leads from the cortex; the active lead on the cortical surface, the indifferent lead from the side of the head, with a heterogeneous mass of tissue between. See text.

the same time. Further sophistication of this line of argument is not profitable without further data.

SUMMARY

This paper presents various lines of evidence as to the character of the electrical responses of the cortex, and as to the position and polarity of such elements. These records cannot be interpreted in the same sense as those from isolated nerve and muscle.

Mechanical and electrical disturbances non-cortical in origin can be obviated.

Results of direct stimulation to an isolated area of the cortex indicate a long refractory period corresponding to the slow rhythm of the spontaneous activity observed.

Tying off the blood supply to an area or isolating it by superficial cuts renders it non-responsive after a few minutes. These procedures demonstrate that cells are involved as well as fibers, and that the leads employed record the responses only of well-localized areas under the electrode on the cortical surface.

Anesthetics depress the activity of the cortex about parallel to the depression of reflex function.

The actual potentials recorded are judged to be those of cells or their specialized processes rather than those of nerve axons.

The series of several responses from the cortex following a single stimulus to fibers of the optic nerve are inferred to be the successive responses of several groups of cells rather than the repetitive responses of one group, although the latter possibility remains if certain qualifying assumptions are made.

The form of the potential records indicates that the reacting elements are directed perpendicularly to the cortical surface, that the impulse passes over them from below, toward the surface, and that certain of them at least are situated close to the surface of the brain.

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DECREASE IN BLOOD VOLUME AFTER PROLONGED HYPERACTIVITY OF THE SYMPATHETIC NERVOUS SYSTEM

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The condition of shock, which was the basal interest for the present study, has been variously designated as surgical shock, secondary or delayed shock, and traumatic shock. It is that condition which comes on at some time after the original injury and is characterized particularly by a small rapid pulse, cyanotic pallor, unquenchable thirst, diminished metabolism, a low blood pressure, and a decreased volume of circulating blood.

The decreased volume of circulating blood in shock was first demonstrated by Keith (1919). Using the plasma-dye method for determining blood volume, he found a consistent reduction in cases of shock and a close parallelism between the degree of reduction and the severity of the condition. His work has been fully confirmed both clinically by Robertson and Bock (1919) and experimentally by Erlanger and Gasser (1919a). It seems to be well established that a reduction in the volume of circulating blood is the centrally important factor in shock.

The mechanism by which this blood is lost from the circulation has been the subject of much research. Following the work of Dale, Laidlaw and Richards (1919), who showed that the injection of histamine could produce shock, with a typical reduction in blood volume, the theory of "traumatic toxemia" was advanced by Quénu (1918) and independently elaborated from animal experiments by Cannon and Bayliss (1919) to account for shock. According to this theory it was supposed that there was formed in injured tissue a histamine-like substance which, on being absorbed, circulated throughout the body producing a dilation and increased permeability of the blood capillaries. In this way, the plasma escaped from the vessels and the corpuscles were stranded in the periphery. In crossed circulation experiments, McIver and Haggart (1923) have obtained evidence of a toxic substance produced on trauma, but Smith (1928) and Parsons and Phemister (1930) were unable to demonstrate any toxic substance coming by the blood stream from an injured area. The mechanism

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of the reduction in blood volume in traumatic shock is thus still left in doubt.

Blalock, Beard and Johnson, (1931) have brought forward evidence to show that the loss of blood and fluid into the injured area is sufficient to account for the reduction of blood volume in shock. This evidence does not agree with that of Cannon (1919b) who was unable, in similar experiments, to find a sufficient increase in weight of the injured extremity to account for the reduction in blood volume. This theory is therefore still not fully proved and besides, it does not account for some of the other factors which are known to cause shock or to aggravate the condition if present. For instance, it is well recognized that pain, fear and cold are of considerable importance in the production of shock. It is impossible on the basis of Blalock's theory to explain how a loss of blood or fluid could result from these conditions.

If we examine all factors which are at present known to produce shock or to aggravate the condition if present, we find that they have *one* physiological action in common. They are *all* adequate stimuli for producing hyperactivity of the sympathetic nervous system. Vasoconstriction, or increased output of adrenin—both of which are indices of generalized sympathetic hyperactivity —has been found to be produced by each one of them, as follows: hemorrhage (Pilcher and Sollman, 1914; Tournade and Chabrol, 1925), cold (Cannon, Querido, Britton and Bright, 1927), fear (Cannon and Britton, 1926), pain (Cannon, 1919a), asphyxia (Cannon, 1919a), infection (Cannon and Pereira, 1924), and low blood pressure (Tournade and Chabrol, 1925).

Vasoconstriction was first found to be present in shock by Seelig and Lyon (1909) and their evidence was confirmed in states of shock produced by a variety of methods by Erlanger and his collaborators (1917). Increased secretion of adrenin in shock was found by Rapport (1922). Since shock is chiefly characterized by a reduction in circulating blood volume and since shock-producing factors stimulate the sympathetic nervous system, the question presented itself, does hyperactivity of the sympathetic nervous system in itself result in a diminished blood volume? This question forms the basis for the present work.

Methods. In a first series of experiments prolonged hyperactivity of the sympathetic nervous system was produced by injections of adrenalin, and blood volume determinations were then made. Control experiments were performed in which sufficient ergotoxine was given to inhibit the vasoconstrictor action of adrenalin. In a second series of experiments blood volume was determined before and during the extreme and prolonged, natural sympathetic hyperactivity resulting from the pseudaffective state after decortication. Again, control experiments were performed in which ergotoxine was given. The pseudaffective state was also studied in com-

pletely sympathectomized animals. Since it was generally believed at one time that shock was due to vasomotor exhaustion with vasodilatation, blood-volume changes were also determined after generalized prolonged vasodilatation resulting from continuous afferent stimulation of the depressor nerve.

Cats were used throughout. In the adrenalin experiments and those on depressor-nerve stimulation they were anesthetized with dial "Ciba," 0.9 cc. per kilo, given by stomach tube. By means of an electric heating pad the rectal temperature was kept close to 38°C. A tracheal cannula was inserted. Blood-pressure records were taken from the right carotid artery by means of a membrane manometer with stiff rubber-tube connections.

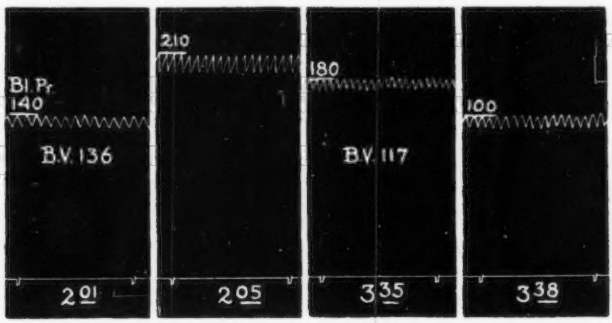

Fig. 1. Effects on blood volume and blood pressure from injecting adrenalin (0.00345 mgm. per kilo per minute) for 90 minutes into a cat. *Dial anesthesia.* Injection started at 2:02, stopped at 3:36. In this as in the following figures, *B. V.* indicates blood volume, *Bl. Pr.* indicates blood pressure, and *H. R.* indicates heart rate. The time is marked in 5 second intervals.

Five per cent sodium citrate was used as an anticoagulant. With the membrane manometer loss of blood resulting from washing out the carotid cannula was reduced to a minimum. It was seldom necessary to wash out the cannula during an experiment. The membrane manometer was repeatedly calibrated during the course of each experiment by comparison with a mercury manometer attached to a side arm.

The adrenalin solution was made up fresh daily by dissolving the tablets (Parke, Davis & Company) in freshly prepared 0.9 per cent sodium chloride, to make a solution of 1:10,000 or 1:20,000. The adrenalin was injected into the right external jugular vein by a constant-injection apparatus. Mineral oil from a Marey flask at constant pressure, flowing through a ground glass stopcock, displaced the adrenalin solution from a calibrated burette.

In the vasodilatation experiments, the central end of the depressor nerve

or the depressor fibers in the left vagus were stimulated, by means of tetanizing induction shocks from a Harvard inductorium, with a current of 3 volts in the primary circuit and with the secondary coil set at 7 to 9 cm. from the zero position.

When ergotoxine was used, the tablets (Burroughs, Wellcome), made up in 0.9 per cent sodium chloride, or the ampules (ergotamine tartrate, Sandoz), were injected intravenously in dosage sufficient to produce a reversal of the effect of adrenalin, as described by Dale (1906). Under dial anesthesia, far larger doses were required to produce this "adrenalin reversal" than under ether or without anesthesia.

Both femoral veins were exposed for the blood-volume determinations. The Cartland and Koch (1928) modification of the plasma-dye blood-volume method of Keith, Rowntree, and Geraghty (1915) was used. Each determination required 1.5 cc. of blood. One cubic centimeter of 1.5 per cent Brilliant Vital Red (Eastman Kodak Company, Rochester, N. Y.) was injected into the left femoral vein and two samples of blood at exactly 2.5 and at 4 minutes after the injection were taken without stasis from the right femoral vein. Exactly 0.5 cc. of blood was drawn into exactly 0.2 cc. of 1.6 per cent sodium oxalate in a calibrated 1 cc. syringe. Hematocrit determinations were made on all three samples of blood and an average of the three readings was taken. A micro-colorimeter (Bausch & Lomb Optical Company, Rochester, N. Y.) was used in order that a minimum dilution of the dyed serum would be necessary. Three-tenths cubic centimeter of dyed plasma plus oxalate was used diluted with 0.6 cc. of distilled water. The standard was made up from the injected dye to contain 0.030 to 0.045 mgm. of dye per cubic centimeter, depending upon the estimated plasma volume. Three-tenths cubic centimeter of undyed plasma plus oxalate was used with 0.3 cc. of the standard diluted with 0.3 cc. of distilled water. The standard was set at 20 and the unknown taken at 2.5 minutes was read. Without changing the standard, the second unknown was then read. The sample containing the greater concentration of dye was always taken as the final reading. Generally it was the sample taken at 2.5 minutes after the injection of the dye.

The limit of the technical accuracy of this method has been set at 4 per cent by Cartland and Koch (1928). The dye leaves the blood stream relatively rapidly. In three experiments, five samples of blood were taken at 2.5, 4, 6, 10, and 16 minutes after the injection of the dye. As can be seen in the graph of one experiment (fig. 2) the values for the concentration of the dye in the plasma fall within a 4 per cent zone of experimental error.

In three experiments, the effect of dial anesthesia on the blood volume was determined. There was a slight fall after the second hour, averaging −2.8 per cent at the end of 4 hours. Since the experiments lasted only 2 hours, this error was ignored.

The injection of 0.9 per cent sodium chloride in amounts equivalent to the fluid injected with the adrenalin over a similar period of time does not alter the blood volume within experimental limits.

Adrenalin injections. It has been recognized since the work of Elliott (1905) that adrenalin mimics the action of the sympathetic nervous system. Prolonged hyperactivity of the sympathetic can therefore be obtained by a continuous injection of adrenalin. Bainbridge and Trevan (1917) were first to discover that it is possible to produce shock by injecting large doses

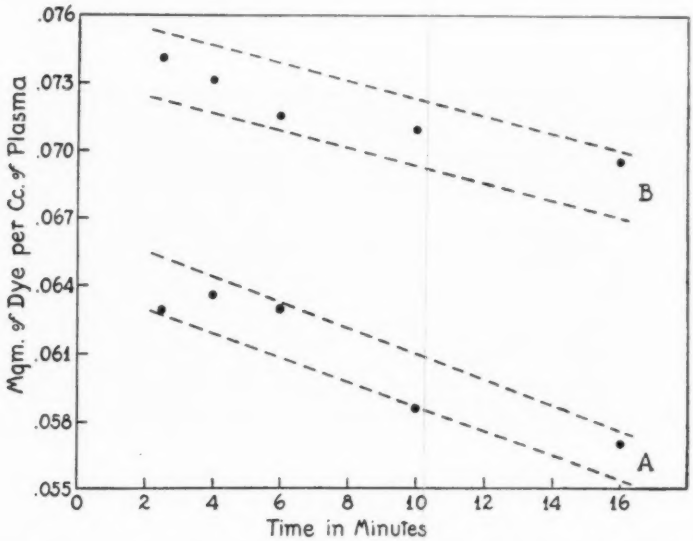

Fig. 2. Concentration of dye in plasma before (A) and after (B) injection of adrenalin (0.006 mgm. per kilo per minute for 90 minutes). The values are found to be within a 4 per cent zone of experimental error shown by the dash lines. The concentration of dye in the plasma indicates a decrease of 15 per cent in plasma volume.

of adrenalin and Erlanger and Gasser (1919b) have demonstrated a decrease in circulating blood volume in the experimental shock produced by injecting adrenalin into dogs. They used very large amounts of adrenalin; 6 to 11 cc. of a solution of 1:1,000 were injected in a period of 21 to 29 minutes, amounts far beyond the maximal physiological secretion.

The rate of secretion of adrenin in response to afferent stimulation has been found by Cannon and Rapport (1921) to be between 0.0032 and 0.0037 mgm. per kilo per minute. In the 17 experiments here reported rates ranging from 0.001 to 0.006 mgm. per kilo per minute were used, the amount of solution ranging from 8.5 to 18 cc. The arterial pressure was thereby

raised to about 200 mm. of mercury. The injection was continued for approximately 2 hours. The initial blood volume was taken just before starting the injection and the final one during the injection.

A reduction in blood volume always resulted from the constant injection of adrenalin in physiological amounts over a period of 2 hours. As shown in figure 1 there is a prompt rise in arterial pressure from 140 to 210 on starting the injection with a gradual fall during the injection to 180 and a fall to 100 on stopping the injection. In this instance there was a reduction in the blood volume of 13.4 per cent, from 136 cc. to 117 cc. The plasma volume decreased to a greater extent than the cell volume as shown by a rise in the hematocrit volume from 31.5 per cent to 37.3 per cent. In a

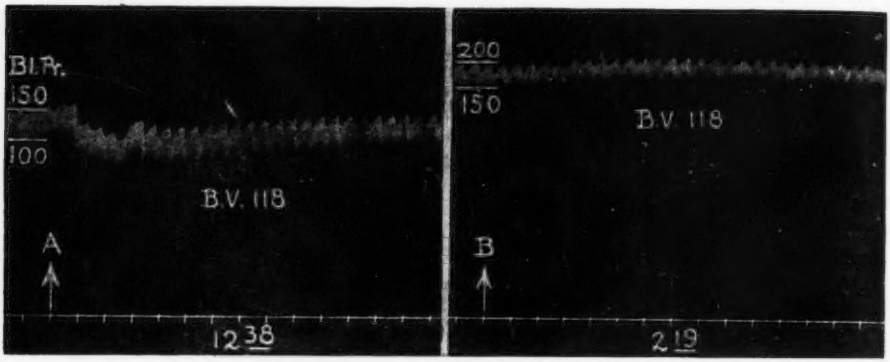

Fig. 3. Effects on blood volume and blood pressure from injection of adrenalin into cat under ergotoxine (8 mgm. per kilo). *Dial anesthesia.* Adrenalin 0.00335 mgm. per kilo per minute for 97 minutes. The arrows indicate (A), the start and (B), the end of the injection.

series of 9 experiments, the average decrease in blood volume was 14 per cent, with variations between −27 per cent and −8.5 per cent. The plasma decreased more than the cells, averaging −17.2 per cent with variations between −29.5 per cent and −10 per cent. The average cell volume reduction was 8.5 per cent with the individual cases varying between −21.5 per cent and +4.8 per cent. The decrease in blood volume was consistently found and was well beyond the limits of experimental error.

That this determination of the reduction in blood volume is not an artefact resulting from changes in the rate of blood flow is shown by figure 2 in which five samples of blood were taken after the injection of the dye before and after injecting the adrenalin. The same degree of reduction in plasma volume after the adrenalin injection is seen when comparing each one of the determinations with the one taken at the corresponding time

before the injection. This reduction, representing 15 per cent of the plasma volume, is well beyond the 4 per cent limit of error shown by the dash lines.

Adrenalin plus ergotoxine. In a series of 8 experiments, ergotoxine was injected intravenously before injecting the adrenalin. The dosage of adrenalin and the amount of solution injected was equivalent to that administered in the series without ergotoxine.

As shown in figure 3, the rise in blood pressure is inhibited on starting the adrenalin injection while there is a transient rise in blood pressure on stopping the injection. The blood volume shows no change. In 8 experiments the average decrease in blood volume was only 1.3 per cent. Generally there was a slight decrease in the plasma volume, averaging −5.6 per cent within the limits of −15 per cent and +3.3 per cent, and a slight rise in the cell volume, averaging 5.7 per cent with variations between −10.8 per cent and +23 per cent.

Pseudaffective state. Exhibition of emotional reactions on appropriate stimulation of a decerebrate cat without anesthesia was first reported by Woodworth and Sherrington (1904). By sectioning the brain at a higher level Cannon and Britton (1925) were able to produce a condition of spontaneous recurrent outbursts of quasi-emotional activity. This condition, which is produced by decortication via the orbits under ether, has been termed sham rage or the pseudaffective state. Extreme hyperactivity of the sympathetic nervous system has been demonstrated to occur. There is a greatly accelerated output of adrenin from the adrenal medulla (Cannon and Britton, 1925), rise in blood sugar (Bulatao and Cannon, 1925), erection of the hairs and rise in blood pressure, all indications of hyperactivity of the sympathetic nervous system. It has been recognized by all who have worked with this preparation that it is short-lived, there being a steady fall in blood pressure and a diminution of activity until death occurs. The average blood-pressure readings in a series of 8 unreported experiments (performed by Cannon and Britton) showed a drop from 137 to 55 mm. of mercury in 90 minutes. It was at Doctor Cannon's suggestion that blood-volume determinations were made on cats in a pseudaffective state to ascertain whether or not in this condition of spontaneous sympathetic hyperactivity a fall in blood volume could be found similar to that found after the injection of adrenalin. If so, it might offer an explanation for the steady drop in blood pressure observed experimentally.

The cats were anesthetized with ether, a tracheal cannula inserted, both carotids ligated and both femoral veins exposed for the blood-volume determinations. In 8 of the 15 experiments, the heart was denervated to furnish an index of the medulliadrenal secretion as described by Cannon and Britton (1925). The adrenin secretion furnished an index of the activity of the sympathetic nervous system. This operation did not materially affect the changes in blood volume to be determined.

At the start of the experiment the blood volume was determined as described before or was estimated in some of the experiments from the values obtained in the remaining cases as 5.7 per cent of the body weight. This figure is approximately the same as that obtained by Harris (1920) who found by the plasma-dye method the blood volume in five cats to average 5.8 per cent of the body weight.

With the vertebral arteries compressed decortication was performed via the orbits and the ether discontinued.

A second blood volume determination, after approximately 2 hours, was made.

At necropsy the exact extent of the damage to the brain and the amount of intracranial bleeding, never more than 2 cc., was noted.

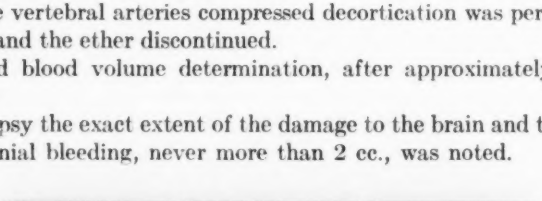

Fig. 4. Effects on blood volume and blood pressure of pseudaffective state for 94 minutes in cat. Heart denervated. No anesthesia after decortication. The cat was bled 10.4 per cent of its blood volume at 11:28.

In every experiment there was a considerable fall in blood volume during the pseudaffective state, coincident with the fall in blood pressure. In the experiment shown in figure 4 the blood pressure has fallen from the original level of 170 to 100 mm. of mercury, while the blood volume has fallen 25.7 per cent, from 144 to 107 cc., the plasma volume 24.1 per cent and the cell volume 28.3 per cent. The average fall in blood volume in 15 experiments was 21.9 per cent, varying between −35.1 per cent and −10.9 per cent, and is quite as much due to a fall in cell volume (25.9 per cent, with a variation of from −39.5 per cent to −11.3 per cent) as due to a fall in plasma volume. The latter averaged a decrease of 19.4 per cent with values lying between −32 per cent and −8.6 per cent.

The rate of the denervated heart (fig. 4) shows that there is a greatly increased output of adrenin. With the adrenals inactivated the denervated heart rate was found to average 156 beats per minute by Cannon and Britton (1925). In one experiment of this series the denervated heart

rate was even as high as 312 beats per minute. Even when the blood pressure in the experiment illustrated in figure 4 is at 60, the rate of the denervated heart is 204, showing that there is no failure of adrenin secretion.

That this measured fall in blood volume is actual and not an artefact can be seen by the reaction of an animal in this condition to hemorrhage. In the case illustrated (see fig. 4), a hemorrhage of 10.4 per cent of the blood volume at 11.28 resulted in a precipitous drop in blood pressure from 100 to 60 mm. of mercury from which the cat failed to recover.

The fall in blood volume does not depend entirely upon the action of adrenin. In a cat in which one adrenal gland had been denervated and the other removed and the liver nerves cut at a previous sterile operation, the blood volume decreased 22.6 per cent from 204 to 158 cc. during a pseudaffective state lasting 90 minutes while the blood pressure fell from 220 to 60, quite as much as in the normal cat.

Pseudaffective state plus ergotoxine. If ergotoxine in doses of from 0.1 to 0.2 mgm. per kilo was injected, the drop in blood volume during the pseudaffective state was largely prevented. In a series of 4 experiments, in all of which the heart was denervated, the average fall in blood volume was only 3.5 per cent with variations between −7.5 per cent and −1.7 per cent. There was an average decrease of 9 per cent in cell volume within the limits of −4 per cent to −13 per cent while the values for the plasma volume varied from +7.8 per cent to −5.9 per cent with an average increase of 1.3 per cent. Correspondingly, the blood pressure was not reduced.

In this series of experiments the action of the vasoconstrictors was inhibited. It is known that ergotoxine blocks the positive action of sympathetic impulses and adrenin on smooth muscle. Ergotoxine has similar effects on the heart, but relatively large doses are required to prevent acceleration of the heart as compared to the doses which will inhibit vasoconstriction (Dale, 1906). The rate of the denervated heart which was always above 200 in each experiment showed that adrenin secretion was still continuing. In spite of this continued high increased secretion of adrenin and all other evidences of the pseudaffective state, such as struggle, clawing, panting, and lashing of the tail, the blood volume was not reduced.

Pseudaffective state in the completely sympathectomized cat. Ergotoxine itself produces vasoconstriction (Dale, 1906) as shown by the high blood pressure of the cat under its influence (see fig. 3). It is difficult to determine the exact dosage and the duration of its effectiveness. In order to eliminate all the vasoconstrictors, complete sympathectomy was performed on three cats by the technique of Cannon, Newton, Bright, Menkin and Moore (1929). After the animals had recovered from the effects of the operation they were used in experiments on the pseudaffective state.

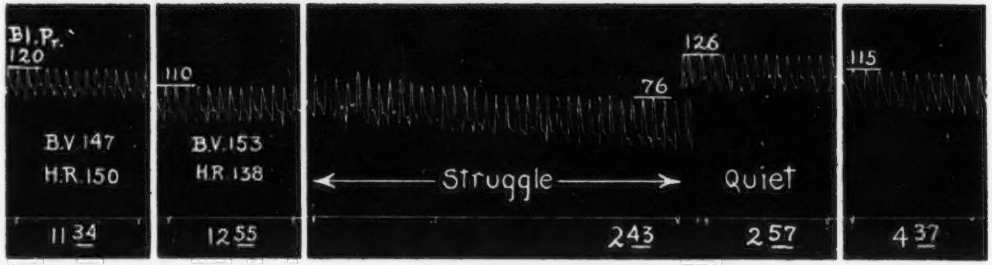

Fig. 5. The effects on blood volume and on blood pressure of the pseudaffective state for 108 minutes in a cat which has recovered from complete sympathectomy. At 2:43 is shown the fall in blood pressure occurring on struggle, with the recovery resulting from a period of quietness.

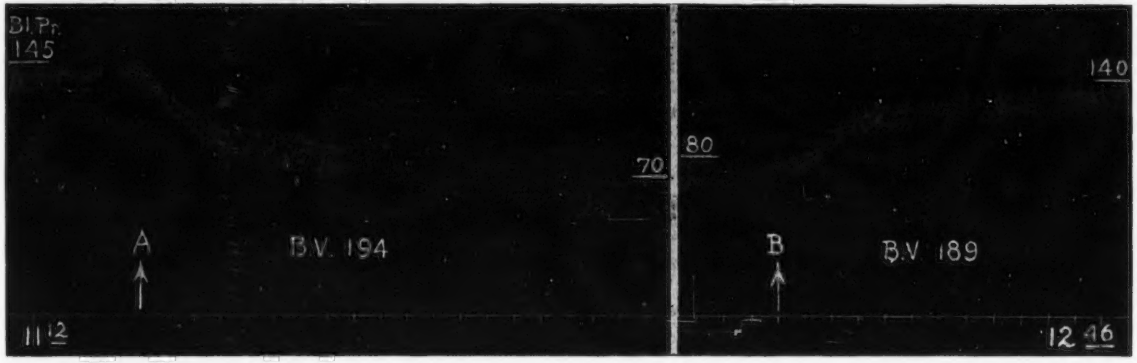

Fig. 6. Effects on blood volume and on blood pressure of continuous depressor nerve stimulation for 95 minutes in cat. *Dial anesthesia.* The arrows indicate (A), the start and (B), the end of stimulation.

As shown in figure 5, in spite of the typical activities of sham rage, the blood pressure was maintained at the original level for five hours and the blood volume, taken at the end of 108 minutes, increased 4 per cent from 147 to 153 cc. There was no rise in the rate of the denervated heart and therefore an absence of adrenal secretion. Also there was no vasoconstriction, as shown by the fall in blood pressure on struggle (see fig. 5, 2:43, and cf. Freeman and Rosenblueth, 1931). In the three experiments there was an average increase in blood volume of 3 per cent, which is within the limits of experimental error. In each case the plasma volume remained almost constant but the volume of cells was increased. The variations in plasma volume were between +7.4 per cent and −7.5 per cent, while the cells varied between −3.1 per cent and +21.5 per cent.

Vasodilatation. Stimulation of the depressor nerve or the depressor fibers in the left vagus was used to produce generalized vasodilation in order to test the theory that shock is due to vasomotor exhaustion. Blood volumes were taken on 5 cats under dial anesthesia at the beginning and at the end of a prolonged period of vasodilation, the result of depressor nerve stimulation.

In the experiment of which the record is shown in figure 6, the blood pressure was reduced from 145 to between 60 and 80 mm. of mercury and was held there for 95 minutes. On cessation of stimulation, the pressure promptly returned to the original level. The blood volume showed a slight fall, 2.6 per cent from 194 to 189 cc., chiefly due to a decrease in the volume of cells, 6.5 per cent. A determination of the blood volume repeated shortly after the end of stimulation showed that the cell volume had returned to its former level.

In 5 experiments the average reduction in blood volume was 2.4 per cent within limits of −7.1 per cent and 0 per cent. In each case there was a decrease in cell volume averaging 7.6 per cent, with no change or even a slight increase in plasma volume (averaging +1.0 per cent).

Discussion. The finding of a decrease in blood volume after a prolonged injection of adrenalin is in agreement with the results of Erlanger and Gasser (1919b). They found a greater reduction in blood volume (averaging 28.8 per cent) than the reductions here reported. They used, however, far larger doses of adrenalin (6 to 11 cc. of a solution of 1:1,000 injected within a period of 21 to 29 minutes, repeated several times) than the physiological amounts here used. A reduction in plasma volume determined by the dye method, averaging 23 per cent in three dogs, was found by Lamson and Keith (1916) on injecting single doses (0.9 mgm. per kilo) of adrenalin. This finding has been confirmed by Nelson and Edmunds (1924) on injecting 1 mgm. per kilo. An increase in blood volume, determined by the carbon monoxide method on injecting adrenalin into experimental animals, has been found by Eppinger and Schürmeyer (1928).

It has been recognized since the work of Boveri (1908) and Steiger (1912) that polycythemia is produced on injecting adrenalin, and Lamson (1915) has shown that this polycythemia caused by injecting single doses of adrenalin is only in part the result of a decrease in plasma volume,—it is also the result of an actual increase in the number of red blood corpuscles in circulation. The spleen has been shown to be the reservoir from which the additional red blood corpuscles are introduced into the circulating blood after the injection of adrenalin (Abderhalden and Roske, 1926). This increase in corpuscles presumably accounts for the increase in blood volume found by Eppinger and Schürmeyer, since they used for their blood-volume determination the carbon-monoxide method, which measures the total volume of corpuscles directly. Prolonged injections of adrenalin were used in the experiments here reported as in those of Erlanger and Gasser in which a reduction in blood volume was found. The increase in concentration of corpuscles indicates that there has been, primarily, an increase in the number of corpuscles in circulation similar to that found with the single injections of adrenalin. With the prolongation of the vasoconstriction from the continuous injection of adrenalin, however, the volume of both plasma and corpuscles decreased.

Ergotoxine or ergotamine tartrate is known to inhibit the vasoconstrictor action of the sympathetic nervous system (Dale, 1906). In the experiments in which this drug was administered intravenously in sufficient dosage to inhibit the vasoconstrictor action of adrenalin, as shown by a partial reversal of the action of adrenalin (see fig. 3), there was no reduction in the blood volume. The blood pressure at the termination of the injection even showed a transitory rise. The dosage of adrenalin and the volume of fluid injected was essentially the same in both series of experiments. It is clear that when the vasoconstrictors were active, a decrease in blood volume occurred, whereas when they were inhibited by ergotoxine there was no decrease.

Injecting adrenalin in such relatively large quantities of salt solution in cats under anesthesia raises the objection that the conditions under which the decrease in blood volume occurred are artificial. In the pseudaffective state, however, after the decortication no anesthetic was used and no salt solution or other foreign substance was injected. The hyperactivity of the sympathetic nervous system was spontaneous. Under these conditions the decrease in blood volume averaged 24 per cent as compared with 20.3 per cent, the average decrease in blood volume found by Erlanger and Gasser (1919a) in experimental shock produced by clamping the aorta or inferior vena cava, handling the intestines or trauma to an extremity. That the reduction in blood volume in the experiments here reported is real is shown by the sensitiveness of a cat at the end of an hour of this condition to hemorrhage (see fig. 4). Normally a cat can recover promptly

from a loss of blood amounting even to 30 per cent of its blood volume, as shown by Cannon (1923). In this case a loss of 10.4 per cent led to a rapid fall in blood pressure from 100 to 60 mm. of mercury, from which the cat failed to recover.

The decrease in blood volume observed in the pseudaffective state is not due to the action of secreted adrenin alone. In a cat in which one adrenal had been removed, the other denervated and the hepatic nerves cut at a previous sterile operation, there was an equal reduction in blood volume and a consequent fall in blood pressure from 200 to 60 mm. of mercury in an hour and a half. In this experiment, although there was no adrenin secretion, vasoconstrictor innervation was still present except in the region supplied by the left splanchnic and the left upper abdominal sympathetic chain.

If the vasoconstrictors are inhibited by ergotoxine, there is still an increased secretion of adrenin, as shown by the increased rate of the denervated heart, but the blood volume is not reduced. It is clear, then, that, even in the absence of adrenin, when the vasoconstrictors are active there is a decrease in blood volume while, on the other hand, even when adrenin is present, the blood volume is not reduced if the vasoconstrictors are inhibited.

The decrease in the total volume of cells, which averaged 9 per cent in this series, may have been due to the reversal of the action of adrenin or sympathetic impulses causing a dilatation of the spleen (Dale, 1906). This dilation of the spleen might lead to a segregation of corpuscles, thus accounting for the decreased total cell volume.

When all vasoconstrictors were removed by previous complete sympathectomy, no decrease in blood volume was encountered (see fig. 5) under the conditions which always led to a reduced blood volume when the vasoconstrictors were present. The persistence of the original level of blood pressure as contrasted with the progressive fall in pressure in normal cats under identical conditions strongly supports the finding of a blood volume which has been maintained at the original level. All signs of extreme sham rage were present in the sympathectomized cat for five hours and still there was an increase in the blood volume, whereas there was always a decrease in the normal cat under identical conditions. The persistence of the normal blood pressure even after five hours of supreme excitement is perhaps the most conclusive evidence that the blood volume was maintained, since it has been shown by Sawyer and Schlossberg (1932) that the sympathectomized cat is extremely sensitive to loss of blood. If there had been any reduction in blood volume the blood pressure would certainly have fallen. This experiment is considered to be crucial.

In order to account for the loss of blood volume in shock, Starling (1918) suggested that the muscle capillaries were dilated and the fluid elements

of the blood escaped from these dilated vessels. It has been maintained by Hartman, Evans and Walker (1928) that adrenalin in certain doses dilates the muscle blood vessels. The effects which have been here obtained showing a fall in blood volume coinciding with sympathetic hyperactivity may have been due to vasodilatation in the muscles rather than to vasoconstriction in other areas.

No persistent decrease in blood volume was found as a result of the vasodilation from depressor nerve stimulation (see fig. 6). The temporary decrease in total volume of corpuscles was possibly due to dilation of the spleen which has been shown by Sollmann and Pilcher (1912) to occur on depressor stimulation. This explanation for the decrease in cell volume is strengthened by the fact that the initial total volume of cells was regained when a determination was made 30 minutes after cessation of stimulation. The blood pressure which was held between 60 and 80 mm. of mercury for 95 minutes promptly returned to the initial level. By depressor stimulation not only are the constrictors inhibited but the dilators are simulated, as shown by Freeman and Rosenblueth (1931). It is thus shown that active vasodilation with inhibition of vasoconstrictors does not result in a decrease in blood volume.

The traumatic toxemia theory of shock, suggested by Quénu (1918), fails to account for the reduction in blood volume found in these experiments. No toxin was introduced in the experiments on the injection of adrenalin and yet the blood volume was reduced. In the experiments on the pseudaffective state, the area of traumatized tissue was largely cut off from the circulation by ligation of the carotid arteries. The protection of the cat from a drop in blood volume by complete sympathectomy would seem definitely to rule out traumatic toxemia as a factor in the reduction of blood volume in these experiments.

It is also impossible to account for the loss of blood volume in these experiments on the basis of Blalock's theory (1931) of a local loss of blood and fluid into the traumatized area. No area was traumatized during the injection of adrenalin. The trauma in the experiments on the pseudaffective state was limited to the cranial cavity with its surrounding rigid walls which prevented swelling of tissues. The same amount of damage was done in the pseudaffective state in the sympathectomized animal in which no reduction in blood volume took place.

It has been shown that active vasodilation with inhibition of vasoconstrictors did not result in a reduction of blood volume. It is impossible, therefore, to account for the reduction in blood volume in the other experiments here reported on the basis of a generalized vasodilation.

It is possible to account for the reduction in blood volume in these experiments as due to prolonged vasoconstriction. A reduced flow of blood in peripheral structures has been repeatedly demonstrated in shock. The

removal of less than 10 per cent of the estimated blood volume of an animal reduced the volume flow of blood through the submaxillary gland by 60 per cent, according to Gesell (1918). With this diminished volume flow of blood there is a relative asphyxia of the peripheral tissues. A reduction in the oxygen content of venous blood in traumatic shock was found by Aub and Cunningham (1920). They attributed this reduction to the slowing of the blood flow. Vasoconstriction has been shown to occur on the arterial side of the capillary net-work by Lewis (1929–31) in Raynaud's disease. This finding has been corroborated by Landis (1929–31). By direct measurements, Landis showed that the capillary pressure during an arterial spasm in Raynaud's disease was between 5 and 8, in contrast to the normal pressure of 20 to 30 mm. of mercury. At the end of the spasm, evidence of increased permeability of the capillaries was shown by the increase in the fluid of the tissue spaces directly observable in the raising of the skin and the blurring of the outlines of the vessels. The subjects often complain of a feeling of tightness after a long spasm. Local asphyxia results in an increased permeability of the capillaries as has been shown by Landis (1928) and by Heimberger (1926). Proteins of the plasma pass with the fluid through the vessel walls into the tissue spaces. As a result of the passage of plasma out of the vessels, concentration of the corpuscles takes place in the peripheral vessels. Evidence for this concentration is found in the relative increase in the red blood count and the hemoglobin content of capillary blood in shock, first described by Cannon, Fraser and Hooper (1919). The internal friction of the blood rises rapidly with the concentration of corpuscles (Trevan, 1918) resulting in still further slowing of the blood flow and eventual stagnation.

The entire process is quite comparable to that described by Mall and Welch (Welch, 1910) in the production of a hemorrhagic infarct by the partial occlusion of a mesenteric artery. They found that when the pressure in the artery was reduced to about one-fourth or one-fifth of the normal (cf. observation of Landis (1929–31), cited above, that capillary pressure in arterial spasm of Raynaud's disease is reduced one-fourth), the smaller and larger microscopic venules and capillaries gradually became more and more distended with red blood corpuscles which accumulated in clumps or in solid columns. This change might become permanent, producing an obstacle to the flow of blood. It is possible to explain the reduction in total cell volume found in the experiments here reported on the basis of vasoconstriction in the arterioles with the consequent reduction in the capillary pressure and the slowing of the blood stream leading to stagnation of the corpuscles in the capillaries.

That the venules have constrictors which are innervated by the sympathetic nervous system was first shown by Bancroft (1898). If venoconstriction occurs, the reduction in blood volume could be due in part to an

obstruction to the flow of blood caused thereby. Mall and Welch found that obstruction of the venous outflow increased the hemorrhagic infarction produced by arterial obstruction. Direct evidence against any venous obstruction in Raynaud's disease has been presented by Landis but it still seems possible that venoconstriction might have played a part in the reduction of blood volume in the experiments here reported.

Finding that vasoconstriction was present in all types of experimental shock which they studied, Erlanger, Gesell, Gasser and Elliott (1917) suggested that the causative factor of shock was "reduced circulation brought about possibly through the action of pain stimuli, and of a certain amount of hemorrhage, on the vasoconstrictor mechanism, this reduced circulation starting in its train such events as follow, for instance, the injection of adrenalin." They did not succeed, however, "either by direct or reflex stimulation of the vasoconstrictor center in inducing in animals a peripheral constriction of the duration that was necessary, in their experience with other methods, to start the animal on the road to shock." It seems clear that, in the experiments here presented, vasoconstriction of sufficient duration has been produced to lower the blood volume and that this reduction in blood volume has been prevented by inhibition of or removal of the vasoconstrictors.

SUMMARY

Prolonged hyperactivity of the sympathetic nervous system, whether resulting from the injection of adrenalin in physiological amounts (see fig. 1) or from the spontaneous emotional activity of the pseudaffective state (see fig. 4), results in a decrease in the circulating blood volume, both fluid and corpuscular elements decreasing.

This decrease in blood volume does not occur in case the vasoconstrictor action of the sympathetic is inhibited by ergotoxine (see fig. 3).

The emotional hyperactivity of the pseudaffective state does not lead to a decrease in blood volume in the completely sympathectomized cat in which vasoconstrictors are absent (see fig. 5).

Prolonged vasodilation with inhibition of vasoconstrictors from depressor nerve stimulation does not result in a decrease in blood volume (see fig. 6).

It is concluded that prolonged vasoconstriction of itself results in a loss of blood from the circulation.

It is a pleasure to express to Prof. W. B. Cannon my appreciation for his suggestions, encouragement and criticisms.

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ACTION POTENTIALS OF THE OPTIC CORTEX UNDER THE INFLUENCE OF STRYCHNINE

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The normal electrical activity recorded from the optic cortex following a stimulus applied to the optic nerve has been previously described. (Bartley and Bishop, 1932, 1933a.) As a further step in the analysis of such responses, the cortex has been experimentally modified by the direct application of strychnine to its surface. Others have previously used strychnine in connection with testing reflexes or sensory behavior. In particular, it has been successfully employed by Dusser de Barenne, who showed (1916) (1924) that very small amounts applied to the cortex of cats and monkeys gave rise to disturbing sensory phenomena. By merely dampening 2 to 3 sq. mm. of the cortex with the drug and blotting off the excess, pain was produced over wide areas of the animal's head or limbs. With reference to "localization of function," the question arose as to how direct disturbance of so small an area should elicit the widespread results that it did. This same question becomes important in dealing with the optic cortex, where in fact it can be demonstrated that a strychninized area becomes a source of impulses propagating into the normal regions. As far as we know, the action potentials of the cortex have never been studied under these conditions. In the present work strychnine was applied to the rabbit's cortex in the same manner as by Dusser de Barenne, after which cathode ray oscillograph records were made of the electrical potentials developed both spontaneously and in response to stimulation of the optic nerve.

PROCEDURE. The animals were prepared as described elsewhere (Bartley and Bishop, 1933a, b) by the removal of the eye and exposure of the optic nerve for stimulation, and the exposure of the contralateral cerebral hemisphere. Upon the cortex, three electrodes were placed about parallel to its long axis as illustrated in diagram J in figure 2. Number *1*, the anterior electrode, was placed well off the optic cortex, as indicated by testing it

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against an indifferent lead from the side of the head while the optic nerve was stimulated. Each of the other electrodes was also tested against the indifferent lead in the same manner to be sure that both were on active regions of the optic area. Each of the electrodes was connected to two selector switches in parallel so that it could be led either to the grid or the ground connection of the amplifier-cathode ray recording apparatus, and any pair could be chosen as leads by turning these switches. The circuit composed of leads *1* and *3* is known as *A*, that composed of *1* and *2* is *B*, and that composed of *2* and *3* is *C*, the second named electrode being always the grid lead.

In the first type of experiment, strychnine was applied to the area immediately under one electrode and the record observed as it took effect. A tiny pellet of cotton was dipped in a 1 per cent solution of the drug and

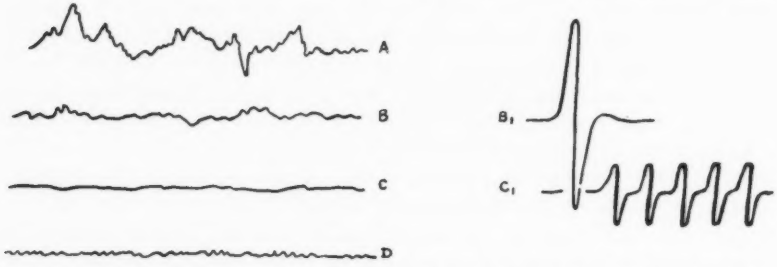

Fig. 1. Diagram showing the progressive change of cortical activity under influence of strychnine. *A* is the normal spontaneous activity of the cortex, *B* and *C* show the progressive reduction and obliteration of the recorded potentials. *D* shows the tiny but regular waves when using higher amplification but under the same physiological conditions as in *C*. *B1* shows the character of the large spontaneous waves that begin to appear as waves in *A*, become reduced to *B*. *C1* shows their relative size as they reach the state of prolonged repetition.

lightly touched on the spot under the electrode. The application was repeated if results did not follow in a few minutes. With the alternative circuits *A* and *B*, we were provided with a control area along with the test one.

Results. If the drug was placed under electrode *3*, the sequence of events was uniformly as follows:

The first alteration by strychnine consists of the diminution of the general level of the spontaneous activity with now and then very large diphasic waves appearing singly or in small groups (fig. 1). Following this the large waves become more numerous and form trains of several groups regularly spaced which die out and reappear periodically. Next the train becomes continuous. The waves in it can readily be differentiated from the normal spontaneous activity of the cortex by their marked simplicity and by their

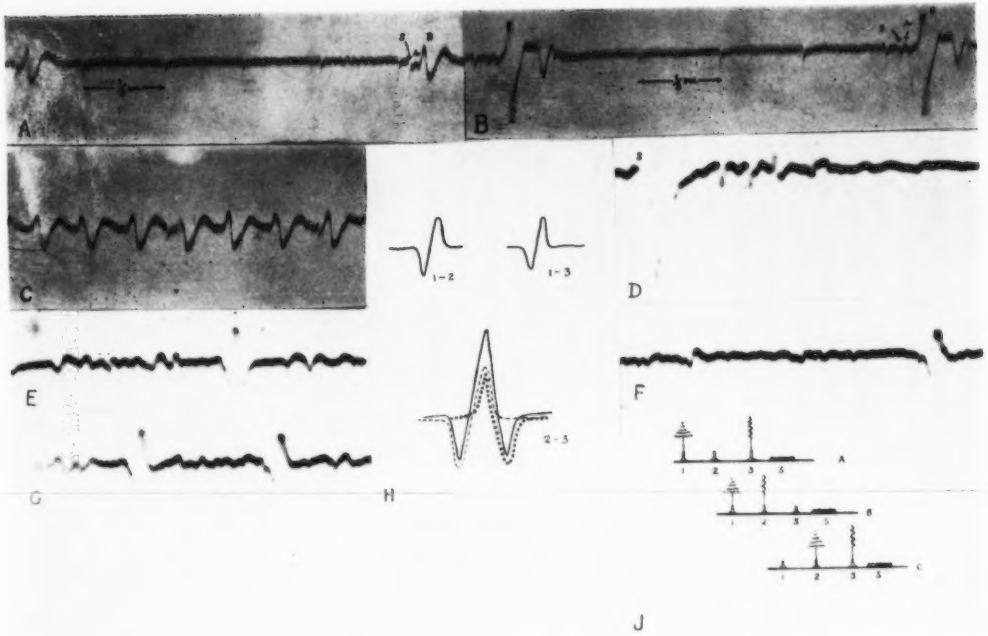

Fig. 2. Records from the optic cortex under the influence of strychnine. The time values for all the records are the same, the one-fifth second interval is shown in A. Record A shows a single response to stimulation; B, a double response; and C a prolonged repetitive response. In records A and B, the two waves of the first response are separated out, and are labelled *A* and *B*. D and E show the responses using points *2* and *3* as leads, under stimulation and spontaneously, respectively. F and G show the spontaneous activity using leads *1* and *3*, and *1* and *2* respectively. Notice that in D, the two small responses following the large shock artifact are triphasic, the first deflection being up in the record, as in the usual case; in E, the large spontaneous triphasic wave shows an initial downward deflection, as do the diphasic waves in F and G; contrary to records A to E. H is a diagram showing the composition of the triphasic wave occurring with leads *2* and *3*, see text. J, diagram of leads on cortex. $\frac{2}{3}$ original size.

great amplitude. The large waves soon become much smaller, break up into groups, and the intervals between the groups become greater, during which intervals there is none of the normal spontaneous activity of the cortex to be seen. Finally only single waves appear or even these fail. There then follows a period of quiescence, after which the whole cycle may be repeated. This occurs again and again; or if it fails, further applications of strychnine cause its recurrence, until finally the cortex fails to respond further, as if from fatigue.

At higher sensitivity of the recording apparatus, during the whole cycle, very small but equally regular sequences of potential waves may be observed. They are in all respects similar to the larger waves, and strikingly different from the quite irregular spontaneous activity of the normal cortex, which, if it were present, would totally obscure these small waves at the sensitivity required to observe them.

Now, if stimulation is given to the optic nerve, at the rate of one per second, and at a time when the cortex is apparently quiescent, instead of the usual normal response made up of a number of components and lasting perhaps a second, a single diphasic wave, identical with the first diphasic wave of the normal record, is all that is elicited. At times even this fails to appear. However, if stimulation is continued, the wave, even if not obtained at first, soon appears at low amplitude and increases to maximal height with repetition of the stimulus. With further stimulation, it begins to repeat after each stimulus by doubling and trebling and so on until it is represented by four or five similar waves in a group, each added wave growing from a small size to maximal by steps. From here, it may abruptly pass into a state of prolonged repetition lasting for as much as several seconds, or on the other hand, it may disappear for a while to reappear spontaneously or after some stimulation. If this sequence does not occur after one application of the drug, two or three are given. From the nature of the waxing and waning of the various effects noted, there seems to be an interplay between conditions making for periodic spontaneous activity and a partial control by experimental stimulation. Complicating this, the brain is probably not in a uniform state of poisoning as experimentation proceeds.

Now if we turn from observing what is happening at the strychninized area to a point nearby on the normal cortex (point *2*), we find a different state of affairs. During the time of diminution of the spontaneous activity at point *3*, we find the usual normal activity continuing at point *2*. It is only later that we find point *2* first quiescent and then repetitive. It thus goes through the same stages as point *3*, but following it by an interval of time. If still more time is allowed, point *2* is seen to be active after point *3* has subsided. When such a stage is reached, an electrode placed *between* points *2* and *3*, namely, near the edge of the poisoned area, records activity.

The central point of the strychninized area has become depressed, but a peripheral region presumably affected less by the strychnine and going into activity later maintains its activity after the central region is fatigued. The effects that we have described obviously spread from the strychninized area, and the lack of the large waves at point *2* during the time that point *3* is first active is not due to inhibition of the rest of the cortex by the poisoned area but to the fact that repeated impulses from it are necessary to establish conduction across the cortex. Further evidence as to the character of this conduction will be discussed below.

Experiments were performed to test for propagation of waves across the cortex. These were made with the same electrode arrangement, the difference being that in this case, the strychnine was applied over an area posterior to point *3* (opposite from point *2*), instead of immediately under point *3*. Enough strychnine was used to largely obliterate the normal spontaneous activity and to set up, if possible, occasional transient spontaneous waves without causing them to become definitely repetitive. Such a condition is the optimal one for observing the potential sign and shape of the waves at the two points, and when the cortex is in this state, it is at its best for the elicitation of single responses to optic nerve stimulation.

The waves in question are first observed at point *3* and appear only later at point *2*. Close examination of the activity at point *2* at times when there are no transient large waves, and at high sensitivity of the recording apparatus, reveals a continuous train of just observable small simple waves. When the larger waves do appear spontaneously at *2*, they are found to be similar to those at *3*, except that they are of lower amplitude at first. When the potentials are elicited by stimulation, they fall later after the stimulus at point *2* than at point *3*. In fact, at a time when point *3* first shows a response to stimulation of the optic nerve, point *2* is inactive in spite of the fact that it was active before strychnine was applied elsewhere. In other words, after applying strychnine to one region of the cortex, other adjacent regions, previously excitable by impulses from the optic nerve, are now accessible to such impulses only by way of the strychninized area.

At points *2* and *3* the potentials are diphasic and generally show an initial positivity indicating in terms of the recording apparatus that the surface of the cortex becomes positive first, or the interior negative. There are times, however, when the reverse is true. The possible connection between this reversal and the potentials of opposite sign in the components of the normal response to optic nerve stimulation will be discussed later.

If the leads are shifted now to arrangement *C* at a time when occasional waves are arriving at both *2* and *3*, as recorded separately, activity occurs under both leads and two types of waves appear in the records. One is the simple diphasic wave recorded previously and the other is a triphasic wave.

The latter appears to be the algebraic sum of the effects as first the one electrode, and then the other, records the cortical activity. From the shape of the triphasic wave and the relation of its parts we may assume that it is produced by potential variations at both electrodes *2* and *3*, each in itself identical to the ones recorded with arrangements *A* and *B*. The manner of superposition is illustrated in *H* of figure *2*. The effect of the potential variation upon the recording apparatus as it arrived under the second electrode would of necessity be opposite in sign to what it was under the first. No triphasic waves appeared in the records while using point *1* as one lead. In comparing leads *A* and *B*, more potential waves appeared at *3* than at *2;* a fact that is consistent with the obtaining of diphasic as well as triphasic waves in using arrangement *C*. The diphasic waves from lead *C* obviously represent the activity at point *3* alone.

We were also able to obtain responses under strychnine following stimulation with arrangements *A*, *B*, and *C*, rapidly enough in succession so that no significant alterations of the responses took place in the time required for taking the three records and checking back on the first. The stimulus artifact here furnished a specific point of reference for the potentials whose sequence was in question. Not only were the responses later at point *2* than at *3*, but the interval between the arrivals at *2* and *3* recorded separately was just sufficient to account for the triphasic waves recorded in arrangement *C*, when the diphasic potentials were added with the sign of one reversed.

In one record from such leads, we obtained diphasic potentials spontaneously after strychnine with the first phase indicating *negativity* at the cortex surface; then following stimuli to the optic nerve, the same leads recorded almost identical potentials but with the phases reversed, that is, surface positive. In all cases, responses following specific stimuli have indicated surface positivity for the first phase whether strychnine was applied or not. In all other cases of spontaneous waves following strychnine, the potentials have had the same form and polarity as have those following stimulation. Further, when stimuli are applied during a period when occasional single spontaneous waves are occurring, if such a stimulus falls just after a spontaneous response, it is ineffective. These circumstances indicate that the same cells are responding in the same general manner, whether due to stimulation of the optic nerve fibers or to a rhythmic pacemaker at the locus of application of the drug. The fact that a simple diphasic wave is recorded at all must indicate that the elements concerned are specifically oriented in the cortex, and that first one pole of each element becomes negative, and then the other pole, as the first recovers. A reversal of sign in the response from a given element would then indicate that the impulse was passing over it in a direction opposite to the usual one, i.e., antidromically. We would hesitate to suggest antidromic condition in the

cortex, even under strychnine, except that so little is known about its physiological behavior that anything at present may be considered as a possibility. Since we have unmistakable records in which one of the five monophasic potentials of the normal response is reversed in sign from the usual polarity, whatever explanations can be offered need not be limited to the case of strychnine poisoning.

Discussion. The diminution in size of the waves making up the normal response was the first change to appear after the administration of strychnine. This does not necessarily indicate a decrease of functioning, and other phenomena that have consistently occurred during the course of experimentation have suggested what seems to be happening. One might first think of a blocking of the activity by strychnine. That the activity of the individual nerve elements is still possible is attested by the fact that as the spontaneous waves with their complicated patterns have about ceased, large transient waves appear first singly, then in small groups, and finally in trains, both in the strychninized area and near by. Further, it is not clear why strychnine at one point should depress *adjacent* regions. During quiescent intervals, with no evidence of there being any of the normal spontaneous activity, one can elicit these large simple waves just referred to, by optic nerve stimulation, indicating that the normal pathway is still accessible.

The alternative, as one finds the original spontaneous waves disappearing, is to think of the poisoning agent as throwing the nerve elements out of phase with each other so completely that their recorded activity cancels. This could happen even in regions adjacent to the strychninized point by asynchronous conduction in many pathways, quite as the larger waves in adjacent regions are shown to be due to conduction in complete synchronism. This is reasonable, for in order to obtain large recordable waves at all in our experiments, great numbers of elements must discharge more or less simultaneously. Just as soon as synchronism is broken down, the conditions cease for any variation large enough to record. The large simple waves that later occur demonstrate that the cells have not been made inactive but that, being thrown out of phase, they can be again coördinated or synchronized by an impulse of considerable size arriving from the periphery, or at least, by a repetitive series of them, or by rhythmic impulses from a pace-maker in the strychninized area.

The two extremes of destructurization and synchronization of the discharge in cortical elements seem to be analogous to extreme individualism ("everyone for itself") and mob action (large groups discharging in simple simultaneity). In the former case, little or nothing shows up in the records; in the latter, large simple waves appear, possessing none of the complications which are seen in the activity of the normal cortex. These simple waves are the very largest of all that we have observed in the cortex of a quiet animal.

It has been stated that at some distance from the strychninized area, as well as in it, reduction of the normal spontaneous response precedes large rhythmic discharges, and that during this period, higher amplification shows many small waves similar to the later large ones in form. We may infer that strychnine causes many cells to become rhythmic but at first asynchronously, and that each cell or group of cells first affected acts as a local pacemaker for surrounding cells; this would keep such driven cells, even at a distance from the strychninized areas, refractory most of the time to impulses initiated by optic nerve stimuli, since the rhythm under strychnine may be higher than can be obtained by optic nerve stimulation. We could thus account for the fact that such an apparently quiescent area loses its ability to respond to stimuli except *via* the strychninized area (as evidenced by the conduction time between the two points) on the basis of greater excitability under the influence of strychnine, but partial refractoriness in the elements driven at above their normal rate elsewhere. The periodic synchronization of all elements in the spontaneous series, or the similar synchronization by means of successive stimuli to the optic nerve, is not difficult to visualize on this basis. Any one group might come to dominate and drive the rest, and stimuli from outside might facilitate putting all elements in the same phase.

The disappearance and reappearance of these waves may be due to either or both of two reasons, namely, fluctuation in the mass impulses coming in from the somatic periphery, or the direct effect of the strychnine on the cortical elements themselves. The disappearance of recorded activity at the strychnine area itself in the final stages of the poisoning, during which time typical strychnine responses can still be elicited at a distant point, namely, point *2*, is a special case of the situation under discussion. Such a fact shows that the changes in question emanate from the drugged region. When they began, they were first detected at the strychninized point and when they ceased they did so first at that point also. In other words, strychnine first excites the cortex, and then either fatigues or depresses it, and the higher the concentration of strychnine (central area of application) the sooner the final stage is reached.

The cortical response to stimulation of the optic nerve changes when strychnine has produced considerable reduction in the normal spontaneous activity. Instead of the response consisting of a series of components and lasting approximately a second, it now consists in a single large element, the analysis of which has shown it to be composed normally of two waves superimposed, one diphasic and a second monophasic wave which is the first of a series of five (Bartley and Bishop, 1933a, b). In the initial stage of strychninization, both these components at times can be detected in the first element when the following waves have dropped out.

We infer that conduction across the cortex from a strychninized area

usually involves each element that responds in the same type of activity as occurs when stimuli arrive at each point separately from the optic nerve. That the latter can occur normally is indicated by experiments (Bartley and Bishop, 1933b) in which a small area of cortex isolated by operation was still capable of responding. Further, there is a limit to the distance through which conduction takes place under strychnine. Beyond 5 to 10 mm. from the point of application, the response to optic nerve stimulation may still be normal and spontaneous waves from the strychninized area die out within this distance. When lateral conduction occurs, it consists of the successive responses of elements at successive points, that is, the diphasicity of each of the potentials gained from points *2* and *3* indicates that they represent activity, similar to the normal, taking place immediately underneath them. The triphasic wave means then that there is activity at each of the two points and presumably at all points between, beginning at successive times. It seems then that what is being recorded at the surface of the cortex at each lead, in this case, is an impulse conducted from below upward as in a normal response to stimulation of the optic nerve, and that the precise nature of the cross connection is still undiscovered.

SUMMARY

The findings reported in the present paper reduce to the following:

1. Strychnine, when sparingly applied to 2–4 sq. mm., of the rabbit's optic cortex, reduces the oscillograph records of the spontaneous activity of large portions of the cortex to almost nothing.

2. During this reduction, large simple waves spontaneously appear, first singly, and then in groups of varying numbers, in and also at some distance from the drugged region.

3. The normal cortical response to stimulation of the optic nerve consisting of five potential waves, is reduced to a single first component in the area noticeably affected by strychnine; this single component can be elicited directly from the changed cortex only at the drugged region, but conducts from here to more distant regions.

4. This first single cortical response to stimulation of the optic nerve finally becomes repetitive under strychninization of the cortex.

5. The responses appearing spontaneously under strychninization and the responses to stimulation of the optic nerve may be assigned to the same cortical elements.

6. The reduction of the record of spontaneous activity under strychnine does not indicate true cessation of activity, but rather such complete asynchronism of the responses of separate elements that their effects counterbalance each other in the record.

7. Conduction of an impulse across the cortex does not involve simple passage of the impulse along continuous pathways as in nerve or muscle,

but consists in the successive activation of elements at successive points, each element apparently oriented perpendicularly to the cortical surface.

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CYCLIC CHANGES IN EXCITABILITY OF THE OPTIC PATHWAY OF THE RABBIT

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It has been shown (Bartley and Bishop, 1933a) that electric stimuli applied to the stump of the optic nerve of the rabbit result in electrical phenomena that may be recorded from the contralateral optic cortex, as a complicated sequence of cortical action potentials. The general procedure followed in the work cited has been employed here, except for details to be noted below. Stimuli repeated at various intervals, from one hundred sigma up to three seconds, in general do not result in identical responses to each stimulus. The question whether the variation in response of the cortex is a random one, or whether some orderly cycle of events gives the appearance of random variation only because of some uncontrolled factor in our procedure, is of fundamental importance, not only in ordering further experimental work, but in interpreting the activity of the cortex itself.

Experiments show that the variations observed in responses to repeated constant stimuli are due to the fact that such stimuli, except at certain critical frequencies, fall at random times in a cyclic variation of cortical excitability. Whether the actual level at which such excitability is varying is cortical or sub-cortical we do not know; it is at some level along the optic pathway. We have good evidence that the cortex itself varies not only in threshold, but in the continuous and apparently spontaneous activity that occurs without specific stimulation of the optic nerve. On the other hand we have some reason to believe that this will not account for all the phenomena observed. For instance, after a maximal response to optic nerve stimulation, the cortex does not appear to be quiescent for the period of refractoriness to a second stimulus. Further, when strychnine is applied to a circumscribed region of the cortex (Bartley, 1933) distant points show a rhythmic activity spreading from the strychninized area into normal regions, at a frequency higher than could be obtained by repetitive stimulation of the optic nerve, and with a constant and not a varying amplitude of response. Finally, the spontaneous cortical activity shows no major fluctuation with a periodicity corresponding to that de-

tected by repetitive stimulation. Apparently the rhythmic variation in the excitability of the pathway is in part at least subcortical, and does not correspond simply to a rhythmic variation in the spontaneous activity of the cortex itself.

Following each effective stimulus to the nerve, two manifestations of activity are observed in the cortex. First, a series of (typically) five monophasic potentials occur at about one-fifth second intervals, the first of the series arising within from five to fifty sigma after the stimulus, depending on the strength of the latter. Second, a single diphasic potential is superposed upon the first monophasic wave of the former series, obscuring its

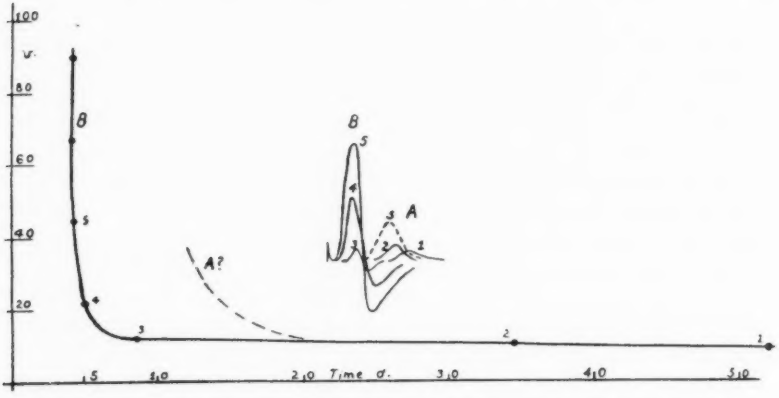

Fig. 1. *Upper:* Diagram of form of potential of first component of the optic cortical record, indicating the low monophasic wave *A* at threshold, occupying positions *1*, *2*, and by inference *5* at successive increases of stimulus strength, and the diphasic wave *B* which falls earlier, and at successive increases of stimulus appears as *3*, *4* and *5*, its second phase obscuring the first wave to appear, *A 5*.

Lower: Plot of time-strength values for the two elements of the first potential complex. The dotted line is the inferred curve of *A*, extrapolated, at a strength where *B* obscures it.

form when above threshold value. It is therefore impossible to observe what is happening to the first member of the monophasic series; but the subsequent responses can be followed, and all members of this series appear to vary in amplitude together. Further, while the thresholds of these two types of waves are different, when a shock well above threshold is delivered, the amplitudes of the two sets of responses vary in a parallel manner with repeated constant stimuli, if not too frequent. This is further evidence that the magnitude of the response is determined not only by the number of fibers stimulated in the optic nerve, but also by the condition of excitability of some critical level of a pathway common to the two impulses. To be

sure, when stimuli follow each other too frequently, only the single diphasic response may occur, the series of monophasic responses being at least too low to be clearly distinguishable, a circumstance suggesting a rather fundamental difference in the significance of these responses in vision; but in most of what follows the intervals between stimuli are relatively long. The amplitude of the larger diphasic potential may therefore be taken as one index of the typical cortical response, (fig. 1) and the details of these responses will be ignored for the present, the whole being discussed in terms of the single diphasic potential.

One major difficulty in this work must be taken carefully into account. Some of the most striking details of the series of events to be described have not been detectable in every attempt to study them. They might thus be taken to be fortuitous occurrences, rather than essential phenomena in the functioning of the visual mechanism. However, in satisfactory preparations the results are so specific and so clear-cut as to leave no doubt in the minds of the observers that they are valid and fundamental evidences of a definite type of functioning. Furthermore, other aspects of this chain of events are constant and reproducible in all rabbits, and the former and the latter types of activity are obviously closely related. We therefore presume that something fundamental which occurs in all experiments is plainly manifested only in some of them because of the complexity of the situation, and venture the opinion that if adequate controls were possible over a nervous mechanism so elaborate as the optic nerve-thalamus-cortex complex, presumably also capable of being influenced by the rest of the nervous system, our experiments would be less equivocal. The best we can say for them at present, however, is that they are not contradictory, and even this at times has somewhat taxed our ingenuity.

In every preparation the following events can be demonstrated. As the stimulus to the optic nerve is increased from below threshold, stimulation at each strength being repeated at three second intervals for at least ten times, a value is reached such that only an occasional response from the cortex is elicited, and never more than a just threshold response. This will be termed the threshold stimulus, as being potentially threshold; it must activate optic nerve fibers that are potentially capable of sending impulses to the cortex, but only occasionally do so. As the strength of stimulus is increased, and more fibers are consequently stimulated each time, cortical responses follow more of the stimuli than previously, and the largest of the responses are greater in amplitude, responses of various amounts being obtained. With further increase, some of these responses become maximal, that is, as large as can be obtained from a stimulus of any greater strength; other responses grade from this value down to nothing. This will be termed a just maximal stimulus even though not all responses to it are maximal. With still stronger stimuli, each is followed by a response, *every* response

being finally maximal. This will be termed a repetitive maximal stimulus. That the variation in response is not to be assigned to variation in the number of fibers activated in the nerve by a constant stimulus may be checked directly by recording the action potential from the optic nerve just beyond the chiasma, and is indicated indirectly by the fact that at certain even shorter intervals the response of the cortex does not vary at all.

If the frequency of stimulation is now increased, say to once per second, a second factor enters. At any given strength, the first response occurs as usual, but subesquent responses are uniformly lower than they would have been at a slower rate of stimulation, even though the stimulus is a repetitive maximal one. After three or four successively lower responses to such a maximal stimulus the rest occur at a new and constant level. This level depends on the frequency of stimulation, with qualifications that will appear presently. For less than maximal stimulation, the responses to a constant stimulus vary, with this new repetitive maximal level as an upper limit. The appearance is similar to relative refractoriness in nerve fibers, where the higher the frequency, the lower the response in any given fiber; but at these frequencies, the locus of such refractoriness certainly cannot be the optic nerve fibers themselves.

Starting at the other extreme, a second stimulus of any strength following a first maximal stimulus is ineffective after an interval of less than fifteen sigma, the two being applied every three seconds. As the interval is increased, a small second response of the diphasic component appears and grows larger, if the stimulus is maximal. If both stimuli are weaker than this, but equal in strength, when the response to the first happens to be large, that following the second will be smaller, but if the first is small or lacking, the second will be large. Two such responses therefore see-saw up and down continuously. This indicates that when, for some unknown reason, a stimulus has been ineffective, it has not left the pathway refractory to a subsequent impulse; that is, the apparently random variations of excitability described above are independent of the impulses sent up the optic nerve by which this variability is made evident.

These events suggest that, insofar as the pathway is concerned which is activated by fibers of the optic nerve having low threshold (activated by submaximal stimuli), there is a spontaneously rhythmic variation in excitability; that the time in this cycle at which the stimulus falls determines whether or not a response shall take place in the cortex; and that if the first of two stimuli falls in a refractory part of the cycle, it does not alter the cycle in phase, and a second stimulus, falling necessarily later in this cycle, must always be effective if the first is ineffective, because by this time these particular elements will have become excitable again. The fact, however, that a stronger stimulus will give a maximal response at any

random time it may be delivered, suggests that if enough fibers respond, the refractoriness of the pathway may be overcome, whatever phase the cycle may be in at the moment. This is not strictly true, as will be shown below; it applies to any first stimulus, but not to properly timed successive ones. An alternate or even a parallel possibility to this last is that different elements are refractory at different times, the fibers of low threshold connecting with elements going through the cycle more or less as a group, those of higher threshold being in a slightly different phase from the first, etc., so that a few elements will be refractory at whatever time the impulses arrive, but on the average, a constant number of elements will be excitable, and therefore a constant maximum response may be obtained. This is a permissible assumption so far as any data we have will indicate. If a submaximal stimulus causes a submaximal response, a maximal stimulus closely following it will always cause a second response, even at a time when a repetition of the first stimulus is ineffective. That is, more of the elements, not activated by the first stimulus, will respond if more optic nerve fibers are activated, even though the elements activated by the first impulse cannot respond again. Further, that all the elements capable of responding at one point of the cortex never can be made to respond to a single stimulus applied to the optic nerve, is shown by experiments with strychnine (Bartley, 1933, l.c.). After having applied strychnine to a small sharply localized area of the cortical surface, repetitive stimulation at one per second will induce responses not only from the strychninized area, but from points at some distance, which are higher in amplitude than any responses ever obtained from the untreated cortex. The evidence (q.v.) indicates that in this case the strychninized area acts as a pace-maker for the adjacent regions of the cortex, which under these circumstances only respond when stimulated by way of the strychninized area; a paradoxical situation, the analysis of which is presented elsewhere. Thus all the elements may respond, instead of only these elements which normally would be non-refractory at the moment of stimulation.

By modification of the procedure of the foregoing experiments, a more specific evidence of rhythmic excitability could be obtained. The optic nerve was first stimulated at the rate of one per second with a submaximal shock, successive responses varying in amplitude as usual (fig. 2). Now the speed of the circuit breaker was altered slowly, until a frequency of stimulation could be found that permitted responses of constant amplitude. That is, the stimulus was brought into constant phase relationship with the cyclic excitability of the optic pathway. If these responses were large, a slight lag of the stimulation cycle caused them to become smaller, even to extinction. In this situation a stronger stimulus would give a response. When the interrupter was timed at a very slightly lower rate than exactly coincided with the rhythm of the optic pathway, or at a slightly higher

rate, the responses varied in a strikingly regular manner, waxing and waning in amplitude by small and definite steps between successive responses, from maximal for that stimulus strength to zero. Apparently at each interrupter cycle, the stimulus fell earlier or later in the nervous cycle by a small amount, an amount depending on the difference between the periods of the two mechanisms. In fact it was extremely difficult to keep the two in exact synchronism, requiring constant slight adjustment of the interrupter motor rheostat.

It was not certain from this procedure whether the cycles corresponded one to one, or whether several cycles of the nervous mechanism might

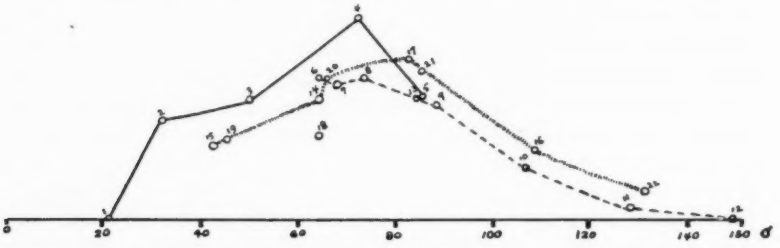

Fig. 2. Plot of second of two responses recorded from a point on the optic cortex, following submaximal stimulation of the optic nerve; amplitude of second response against time of second stimulus after the first. Numbers beside points indicate the order in which readings were made; the curve was repeated several times with the readings staggered as indicated by numerical order. Each point plots the highest amplitude in a series of ten or more responses with a given interval between stimuli; the interrupter turned once per second, and a pair of stimuli were applied to the optic nerve every three seconds. The two stimuli were equal in strength, variation in amplitude of successive responses with the same stimulus interval being assignable to variation in the phase of the spontaneous cycle of refractoriness at which the stimuli happened to fall. During the first twenty sigma the pathway is completely refractory to a second stimulus equal to the first, but if the second had been made stronger than a first, activation of other fibers in the nerve having a higher threshold would have given a response in this interval, and a higher response elsewhere than was observed. This whole curve corresponds to the interval *xyz* of figure 3 *b*, q.v.

correspond to one of the interrupter wheel. Two stimuli per cycle were therefore employed, the interrupter still synchronized with the cortex for a single stimulus. By applying the second stimulus at varying times after the first, keeping the first in constant phase with the cortex, an interval was found such that the second of the two responses was of equal height with the first (fig. 3, a and b). If the first was in the "maximal" part of the cycle, the second was smaller on either side of the critical interval; if the first was minimal, the second was larger when thus out of phase. The critical adjustments required indicated again that the cortical cycle was not influenced by the stimuli employed. The cycle of rhythmic excitability

in the optic pathway so detected was somewhat more than one-fifth second, and varied in different preparations from one-fifth to one-third. Not all animals gave such quantitatively clear-cut results, but in all attempts the most constant series of responses was obtained at a critical frequency of stimulation, *with submaximal stimuli.*

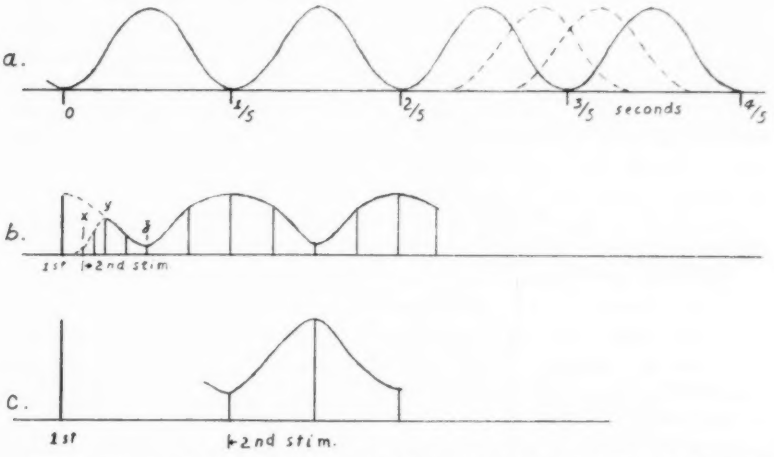

Fig. 3. Diagram to indicate the cyclic fluctuations in excitability of the optic pathway to submaximal and maximal stimulation of the optic nerve. *a*, cyclic variation in refractoriness for fibers of lowest threshold, or for the pathways they lead to. Pathway accessible at 0, ⅕, etc., refractory half way between these points. It is inferred that pathways of higher threshold are out of phase with those of lower threshold, as indicated by dotted curves, such that to a stronger stimulus a response may be obtained even when those paths responding to a weaker stimulus are refractory.

b. Diagram of responses to second of two equal submaximal stimuli when the first falls in the non-refractory part of the cycle. For fifteen to twenty sigmas the pathway is left completely refractory by the *first response;* it is partly refractory after that (from x to y and even from y to z), due to the cyclic variation going on independently of the stimulation (represented by the curve of fig. 2), and for this reason the maximum at y is not as high as the first response. Beyond z the response rises again to reach a second maximum practically as high as the first response, one cycle after the first stimulus, in the phase of non-refractoriness, and so repeats. The cycle of refractoriness is not altered by the stimulation.

c. Responses as in *b*, but both stimuli supermaximal. Apparently a sufficiently strong first stimulus activating a sufficient number of fibers can render all the pathways refractory; and when this occurs the pathways are refractory again one nervous cycle later, but after one and a half cycles the second stimulus may cause a second maximal response. Such a strong stimulus has thus thrown all pathways into the same *phase*, and thereafter the phase relationships impressed upon the system by the stimulus are maintained for some time, contrary to the case of weaker stimulation, (*3*, *b*,) where the phase relationships cannot be shown to shift from their spontaneous rhythm.

In one particularly clear-cut experiment, the interrupter was speeded up until definitely out of step with the cortex, and since the interval between the two stimuli in each cycle was changed in consequence, this was again adjusted to its previous value of exactly one nervous cycle. Now a single stimulus of submaximal value gave varying responses, but when both stimuli were applied at each interrupter revolution, both responses were of the same amplitude, whatever that value happened to be. Again when nearly in time, the responses, both first and second in this case, increased and decreased slowly and rhythmically as the cycles of interrupter and cortex shifted in and out of phase. The response is determined therefore not primarily by the frequency of stimulation through causing refractoriness or fatigue, but by the phase of the nervous cycle at which the stimulus is applied. This fact is emphasized in view of what follows.

If stimuli, of submaximal value in the preceding trials, are increased to fully maximal values, this series of events is materially altered. It will be recalled that such maximal stimuli call forth in general maximal responses; even when applied at one per second frequency the amplitude of response, although lower than with slower stimulation, usually becomes constant, or varies but slightly. When a second stimulus is applied at a shorter interval than one second after a first, this is not true. The former experiments with two stimuli per cycle were repeated with strong stimuli. In one experiment, at the interval corresponding to one nervous cycle as tested by submaximal stimuli, a second *maximal* stimulus gave no cortical response at all (fig. 3, c). This interval was now prolonged, until at one and one-half cycles as determined previously, the second response became nearly as large as the first, and at greater intervals became again smaller. This occurred for any one pair of stimuli, applied after a period of rest, and without regard to the phase relationship of interrupter and nervous mechanism. In other words, it appears as if a *maximal* stimulus excited all or at least a constant number of elements of the pathway regardless of the phase they were in, and in so doing, *forced them all into the same phase*, rendering them refractory thereby, so that at the end of one complete cycle they were all again refractory, but after one and one-half cycles, all were again irritable.

Phenomena similar to these, and obviously having the same basis, could be produced by various manipulations of the two shocks, and some of them were too complicated for analysis at the present stage of our information, and not all could be repeated on different preparations. They are referred to here as giving further and striking evidence not only of definite rhythmicity in the optic pathway, but as indicating that by strong stimuli this rhythm can be changed in phase by the stimulation of the optic nerve. For instance, two maximal shocks were spaced at an interval corresponding to one and one-half nervous cycles, both giving maximal responses as above.

The interrupter was run at three and one-half nervous cycles per revolution, so that at successive revolutions a given stimulus would be alternately in and out of phase with the optic mechanism. Now during the first interrupter cycle after the stimulating circuits were closed, both responses were maximal; at the second cycle, both were just visible, and equal; and this alternation repeated indefinitely, and with successive trials. Further, changing the speed of the interrupter, faster or slower, did not throw the responses out of phase, over a range that with sub-maximal stimulation always throws the two mechanisms throughly out of synchronism.

In other words, not only could the nervous mechanism be driven, but the rhythm so set up maintained the phase imposed upon it for at least the greater part of a second, that is, during several cycles of the nervous activity during which stimuli were not given it. Each effective stimulus apparently set a pace for the rest during one interrupter cycle at least, and another stimulus arrived before the two mechanisms were allowed to get out of phase enough to interfere with the regularity of the results. In other experiments, instead of presence and absence of responses, regular variations in amplitude of lesser degree were obtained.

Discussion. Several major conditions are presented here which seem to us to form a scaffolding within which an explanation of the functioning of the visual pathway must somehow be constructed. No pretense is made that even the scaffolding is complete. First, while the retina and the cortex are presumably connected in a one-to-one point relationship, that is, unit areas of the retina are projected on the cortex by means of potentially simple pathways, these conductile elements are not entirely independent of each other. Activation of one group of elements may affect the time of arrival of impulses at the cortex over another group, even without the mediation of retinal interconnections. Secondly, there is some level at which a rhythmic variation in irritability takes place, apparently spontaneously, but this rhythm can be driven if enough elements are activated at once; again evidence that one element of the pathway can affect the activity or excitability of others. Third, there are connections available, from one element of the cortex to others, accessible under the influence of strychnine even at a distance from the strychninized area, and presumably normally occupied in the "spontaneous" activity shown by the cortex. Whether there may be such connections by way of the thalamus we have ourselves obtained no evidence, but we have evidence from direct stimulation of the cortex (Bartley and Bishop, 1933b) that they are available at the cortical level. Added to these facts, the further data that two more or less independent pathways exist from optic nerve to cortex, as indicated by two distinct potential components (Bartley and Bishop, 1933a), and that one of these at least can be activated only at such a rate of stimulation that it could not reasonably account for the immediate facts of vision, and

we have the major limitations that seem to be imposed by this work so far on the elaboration of an analysis of visual functioning. Even this takes no account whatever of the complications of the retina.

It is obviously futile at present therefore to attempt to "explain" any significant aspect of vision in terms of the records of cell activity that we have obtained so far, and we disavow any such purpose in the remarks which follow. However, it is proper to enquire what limits the facts of vision impose upon the interpretation of our records of nervous phenomena, as well as the other way around. One way of doing both is to construct a hypothetical system that might work if it really existed, one consistent with known facts, but with the gaps that must inevitably appear filled in by figments of the imagination. Such a working hypothesis then suggests further experimental tests, and is perhaps the more stimulating for being constructed so largely out of pure moonshine.

A primary requirement of such a system is that there should be possible, not only a punctate representation of retinal elements on the cortex, but that this representation should be sensibly continuous in time. That is, it is desirable that each unit area of the retina (permissibly including a number of sense endings) be able to activate the cortex at least as frequently as the rate at which separate light stimuli can be differentiated. We do not know what fusion frequency a rabbit has, if any, but may assume it is of the same order of frequency as in man, or possibly lower.

The simple diphasic potential wave recorded from the cortex can follow the second of two stimuli to the optic nerve when the interval is not over fifteen sigma, but the second response is low, and a third stimulus after the same interval is ineffective. Spaced at a reasonable fusion frequency (10 to 20 per second), each would be effective when it did not fall in a refractory point of the cycle of the optic pathway. This potential might thus signalize the first response by the cortex in the simplest form of sensory perception. We have found no evidence of cortical activity that specifically follows as a continuation of this first step; but the reverberations through the optic or other cortical regions of such an impulse, presumably attenuating in intensity as it disperses in space, might easily be lost among the spontaneous discharges so continually in evidence. We need not even assume, however, that it goes any further; perhaps the modification by such an impulse of the cortical activity continuously taking place is in itself the visual act. Presumably the retinal discharges may be higher in frequency than the cortex can follow; the summation of such discharges at lower levels with a change in frequency of the response has been but briefly enquired into, but we have some evidence that a second stimulus to the nerve which fails to give a second separate response from the cortex may increase the amplitude of a first response. The limit of fusion frequency may be imposed by sub-cortical or cortical synapses, at the same level as the refractory

phenomena we have studied, and the variation of fusion frequency with intensity of stimulation has an obvious analogue in the summation phenomena we have met with. An increase in number of fibers stimulated lessens the shortest interval between responses, and an increase in the frequency of stimulation seems to increase the number of cortical elements responding.

The other response obtainable from the cortex, a series of monophasic waves, cannot be made to repeat at a frequency that corresponds to the more obvious events of visual experience. However, visual events are not all obvious; in particular there is demanded some machinery by which the activity of the thalamus as a visual level in itself may be correlated with the activity of the cortex. As the more complicated processes require more time for their accomplishment, so they could be handled by mechanisms more flexible and more complex, but less quick-acting. At least this succession of responses has some of the characteristics that seem appropriate to such complex activity.

We have been able to record specific responses from the cortex only by causing many elements to respond at the same time, and thus to summate their results. Inferences from the results of this simple procedure to the normal case when impulses arise from the retina, will be precarious ones. It is not necessary to suppose that even from a unit area of the retina, only a unit pathway leads to the cortex. There may be several such pathways in parallel, and the cyclic alteration of excitability that we observe may affect them in rotation, leaving some pathway always accessible. In particular, it is too striking to be fortuitous that all of the optic nerve fibers of lowest threshold lead to elements that are approximately in phase with each other, but are out of phase with elements whose fibers have higher thresholds. Such grouping of fibers according to thresholds is reminiscent of the conditions in peripheral nerves (Heinbecker, Bishop and O'Leary, 1933), where fibers serving a common function are usually thus set off from fibers serving other functions. The optic nerve itself is being investigated from this point of view.

The only assumption required to allow continuous representation in the cortex of unit retinal areas is that each such pair of loci, in retina and cortex, be connected by several fibers having different thresholds to electrical stimuli; this threshold difference, whatever other significance it may have, connoting according to our findings a difference in phase of cyclic excitability of the pathways. An impulse from the retinal sense organs might then avail itself of whatever pathway was open at the time and, by means of cortical cross-connections, affect the same cortical locus over any pathway of a group.

In general, it is not necessary to infer that each individual impulse travelling up a fiber from the retina arrives as a unit impulse at the cortex, and

registers there as such. Rather we would look upon the cortex as being in constant activity, the physiological activity of the whole network of neurones bearing some direct relationship to the "present state" of the animal's complex behavior which is sometimes referred to as his "mental state." Impulses coming in from the periphery, by one route more or less directly, by a second which reflected or interpreted the general state of activity of the thalamus as conditioned or modified by such impulses, would then take effect by modifying the cortical activity continuously going on, and the *change* in the whole picture of such continuous activity, rather than the specific set of impulses sent in, would correspond to visual perception. Such activity would then tend to revert, gradually and possibly with rhythmic disturbances still persisting, to its previous state of activity. Failure to return completely would correspond in a sense to memory; rhythmic disturbances persisting after the exciting impulses ceased would have an analogy at least in such phenomena as after-images.

This imaginary picture already far outdistances our experimental findings, but that is the privilege of hypotheses in general. We may at least rest assured that such a picture does not err on the side of complexity, however improbable as fact.

The writer wishes to acknowledge the assistance of Dr. S. Howard Bartley in this work.

SUMMARY

Successive equal stimuli applied to the optic nerve of the rabbit in general evoke from the contralateral optic cortex responses of variable magnitude, unless the stimuli are very strong, or unless they are timed at certain critical intervals.

The shortest critical interval of one-third to one-fifth second presumably corresponds to a cyclic spontaneous variation in the accessibility of the optic pathway, of the character of partial refractoriness, but independent of the stimuli applied. Strong stimuli (activating many fibers of the optic nerve), seem to be able to activate such pathways even when partially refractory, as if by summation at any one element of the effects of the impulses arriving over many optic nerve fibers. This and other similar evidence suggests that the projection pathway from one point of the retina to a corresponding point of the cortex is multiple, and subject to the influence of impulses over other similar pathways.

Various experimental and hypothetical considerations are advanced in an attempt to construct a workable scheme that would reconcile these facts with vision.

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THE EFFECT OF THYROID FEEDING ON TISSUE RESPIRATION

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In recent years, a number of workers have attempted to demonstrate an increase in the respiration of isolated tissues under the influence of the thyroid hormone. In general, thyroxin or whole thyroid preparations have been added to tissues in vitro, or the tissues have been removed after administration of thyroid to the intact animal. The results with both procedures have been conflicting.

Without reviewing the older work, adequately treated in several papers (Paasch and Reinwein, 1929; Weil and Landsberg, 1929; Abelin, 1930; Robles, 1931), some of the more recent material may profitably be considered. This is fairly consistent in negativing an increased respiration of tissue brei or slices or erythrocyte suspensions following in vitro additions of thyroxin (Rothschild, 1930; Horn, 1930; Hopping, 1931); although increased acetaldehyde formation by muscle (Simon and Blazso, 1929) and accelerated liver autolysis (Simon, 1929; Weil and Landsberg, 1929) have been reported. A reversible change in the fatigue curve (on submaximal stimulation) of frog sartorius has also been reported to follow the addition of thyroxin to the medium (Rosemann, 1931). In view of the long delay between thyroid administration to the intact animal and a subsequent increase in metabolism, such negative findings on the respiration of isolated tissues exposed for short periods to thyroxin are hardly surprising.

Still, many workers have seen in these negative results evidence that the thyroid hormone does not directly affect cell metabolism in vivo, but evokes an increase only secondarily to organismic influences. There have been suggested particularly 1, an increased sympathetic action on the body tissues, either by sensitizing the cells to normal sympathetic discharges or by increasing the discharges (Paasch and Reinwein, 1929), and 2, an increase in protein split products, notably tyrosin, (themselves, of course an index of metabolic change) which then stimulate cell metabolism (Dresel, 1929). Both views have even been combined into an inclusive theory (Weil and Landsberg, 1929). A delayed increase in basal metabolism might, however, as well depend on slow changes induced by thyroid in the tissue cells themselves. The view that thyroid hormone leads to a gradual increase in the cell respiratory catalysts, as urged especially by

Dye and his colleagues (Dye and Waggoner, 1928; Dye and Maughan, 1929a, b), belongs in the latter category. Unless nerve or humoral influences have a definite after-effect, the respiration of tissues isolated from hyperthyroid animals should remain normal; whereas if the activity of the individual cells is modified, their respiration should increase for a few days after thyroid administration is begun and remain elevated for a time after it is discontinued. Of course, both types of influence might summate in the intact animal.

The recent evidence, despite contradictions, does favor the conclusion that tissues from thyroid-treated animals exhibit an increased oxygen consumption (and little change in glycolytic activity). Rohrer (1924) reported such results on several tissues and Hopping (1931) on alligator blood, and Robles (1931) observed a progressive increase in the respiration of mouse liver, kidney and skin slices as the time between feeding thyroid (plus some anterior pituitary) and removing the organs was lengthened, up to fifteen days. Conversely, tissues from cretin rats, dogs and sheep showed in vitro a diminished respiration (and oxidase activity) (Foster, 1926; Dye and Waggoner, 1928; Dye and Maughan, 1929a, b). Further, the beat of the completely denervated heart is augmented in the thyroid-fed dog as much as is the innervated one (McIntyre, 1931), and the oxygen consumption of a heart-lung preparation from thyroid-fed rats is increased over normal (Dock and Lewis, 1932). The beat of nerveless embryonic chick cells in culture is also increased subsequent to addition of thyroxin (Markowitz and Yater, 1932).

The present experiments were undertaken, before some of the more definitive recent work appeared, in the hope of deciding whether or not tissues from animals rendered distinctly hyperthyroid for a considerable period displayed in vitro an accelerated respiration. Our results on the dog are in harmony with recent ones on mice and rats in showing such an increased oxygen consumption. Evidence as to the normal control of thyroid secretion will also be presented.

METHODS. Young dogs were fed 0.6 gram of desiccated thyroid per kilo per day for three to six weeks. At the end of the feeding period, when marked hyperthyroid symptoms were present (see McIntyre, 1931), the animal was lightly anesthetized with ether. About 40 cc. of blood were drawn into centrifuge tubes and at once centrifuged. A clot formed during the spinning, but after the cells were carried down. From the pale yellow clot in the upper third of the tube clear serum was easily expressed, and this was used as the medium for suspending tissues. The desired organs were then exposed, thin slices cut with razor blades, weighed on a torsion balance and placed in the serum. Except for a short exposure to room temperature during weighing, the tissues were kept at 37°C. After a run, they were dried (at 110°) to constant weight to obtain the percentage

of water. Except for skeletal muscle, thin preparations were easily obtained with very little cell destruction. The muscle slices were made parallel to the fiber direction to minimize such injury, but even so a very rapid fall in oxygen consumption has led us to discard our observations on this tissue. Normal animals matched in age and size with the hyperthyroid ones were used as controls.

Oxygen consumption was measured in Warburg manometers of small volume (approximately 3 cc.), shaken about a vertical axis (Gerard, 1931) in a bath at 37°C. In each vessel were placed 0.7 cc. of serum, 20 to 150 mgm. of fresh tissue (depending on relative respiratory rates), and 0.1 cc. of normal NaOH, and the mounted vessel was flushed with oxygen. The alkali present would, of course, remove CO_2 slowly from the serum as well as from the gas space, gradually raising the pH. We have found in other work, however, that the pH does not rise above 8.0 to 8.5, and controls with added buffer or bicarbonate have paralleled the results in untreated serum. Further, any progressive changes would affect alike the thyroid and control tissues. In fact, the measured oxygen consumption of most tissues fell progressively during the run, usually lasting two and one-half hours, but even larger falls occurred in phosphate Ringer and no regular differences in the fall in serum appeared between the normal and experimental groups. The direct measurement of oxygen consumption, when it can be applied, is preferable to the differential solubility method (Warburg, 1924), since the chance of error is much less with the former.

Results. The average rate of oxygen consumption during the 150 minute run is given for each tissue and each animal in table 1. The figures represent cubic millimeters of oxygen consumed per hour per gram fresh weight. In table 2 are reproduced results of a typical experiment, indicating the change in oxygen usage with time for individual tissues. The percentage dry weight of the tissues used, given in table 3, was not greatly nor consistently altered as a result of the hyperthyroidism, and oxygen consumption per unit dry weight parallels that for fresh weight.

The experimental series is small (unfortunately several of the hyperthyroid animals died before they could be used and circumstances prevented a repetition), but we believe the results to be entirely significant.

The data on each tissue alone are suggestive but not convincing (except, possibly, for the changes in the thyroid). The further fact that the mean respiration of liver, heart and nerve, despite marked tissue differences, has been increased in each case almost exactly 25 per cent by thyroid feeding lends considerably more weight. But the clinching point is that the thyroid gland respiration under identically the same conditions was decreased almost 30 per cent in the fed group. A random variation in the individual animals composing each group might happen to give tissues of the fed group a higher mean value, but it could hardly lead to the same

TABLE 1

TISSUE	THYROID		VAGUS		LIVER		AURICLE	
	Fed	Control	Fed	Control	Fed	Control	Fed	Control
Oxygen consumption (cmm. per gm. fresh weight per hour; Q_{O_2})	580*	790	215	195	2680	1870	310	430
	520	780	245	195	1800	1570	390	200
	505	550	165	132	1750	1540	520	310
	410		220		2050		350	
Mean	505	710	220	175	2070	1660	390	315
$\frac{\text{Fed}}{\text{Unfed}}$	0.71		1.26		1.25		1.24	

* The values for organs from one dog are in the same row.

TABLE 2

Control dog, June 10, 1931

TIME INTERVAL	MM. CHANGE IN MANOMETER READING PER 10 MINUTES			
	Thyroid	Vagus	Liver	Auricle
10:45–10:55	14	12	43	46
10:55–11:05	11	13	38	37
11:05–11:15	13	13	36	38
11:15–11:30	12	13	36	32
11:30–12:00	11	13	36	32
12:00–12:45	10	12	35	32
12:45–12:55	10	11	35	30
12:55– 1:15	9	11+	33	30
Cmm. per hr.	18.7	23.0	60.0	59.1
Mgm. of tissue (fresh)	23.7	117.7	32.2	137.4
Q_{O_2}	790	195	1870	430

TABLE 3

TISSUE	DRY WEIGHT (PER CENT OF FRESH)	
	Fed group*	Control group
Thyroid	20	24
Vagus	27	30
Liver	25	25
Auricle	23	22

* Total weight loss in the fed group, from the start of thyroid feeding, averaged 29 per cent.

increase in three and an even greater decrease in the fourth. (The same consideration disposes of the possible objection that thyroid-fed tissues were suspended in thyroid-fed serum, normal in normal, thus referring respiration differences to an altered composition of the serum itself.)

Though a statistical analysis is hardly justified by the few data, it is interesting that an application of Fischer's (1926) equations for small numbers gives the following probabilities of the observed differences in the fed and unfed groups occurring by chance sampling (P) as: thyroid, < 0.05 (i.e., less than one chance in twenty); vagus, 0.15; liver, 0.11; auricle, 0.31. The standard formulae (Meller, 1922) further yield the result that the ratios of values for fed thyroids, expressed as a fraction of their controls, to fed other tissues as a fraction of their controls differs from unity by five times the probable error of the ratio. It is worth noting also that the auricles, which show the greatest variation, had been denervated in the experimental, not in the control series.

Discussion. Our own findings, together with other recent work, confirm the view that the thyroid hormone acts upon cells to increase their metabolic activity. In the intact organism a direct action on tissue cells may well be enhanced by secondary stimulation via nervous or humeral routes. The evidence for these latter is far from conclusive, yet some secondary action might be expected. For, if the hormone stimulates cells in general, this will include neurones, bone-marrow, etc., and their increased activity would, via these secondary correlation routes, in turn influence other cells. To emphasize these secondary factors is, in our opinion, incorrect.

The further analysis of the modus operandi of the incretion on cell respiration is not possible from our results, which, however, are in harmony with Dye's findings and interpretations. A very similar view of nerve degeneration, as depending on a gradual loss of cell catalysts, has been expressed by one of us (Gerard, 1932), and thyroid feeding does hasten nerve regeneration (Maekawa, 1923; Cook and Gerard, 1931).

Possibly of greatest significance is the finding of a decreased respiration of the thyroid gland from animals fed thyroid. It has long been known (Loeb, 1919; Abelin, 1930) that after partial thyroidectomy the remaining gland shows histological evidence of hypertrophy. This overgrowth can be suppressed by thyroid administration, and even a normal gland is changed into a state of partial involution in the same way (Fordyce, 1912; Loeb, 1920). McJunkin and Roberts (1932) have just reported an entirely analogous decrease in mitosis in the islet cells of the developing pancreas from animals treated with insulin.

The decrease in metabolism now found in thyroids of thyroid-fed animals is a comparable but not entirely equivalent effect. The respiration is expressed per unit weight of the gland, and a mere change in total gland size would not affect its value. The water content of the gland has

slightly, possibly not significantly, increased, but a relative increase in colloid may be assumed (Fordyce, 1912; Krogh, Lindberg and Okkels, 1932) so that we can not surely conclude that the same number of cells in an involuted gland have less metabolic activity than in a normal one. This is, however, very probably true and implies that the individual secreting cell is caused to pass from a state of physiological activity associated with increased respiration and presumably secretion, to one of physiological rest or lessened activity by the presence of an excess of thyroid hormone in the blood. This strongly supports the view that the level of hormone in the blood reaching the gland is an important means of regulating its activity; though control via intermediary effects is, of course, not excluded. Spontaneous hyperthyroidism would seem to exclude such a mechanism; but just in this disease the regulatory control has broken down, hence the overproduction of hormone in the presence of an excess of itself, and it might well be the mechanism under consideration that has failed.

SUMMARY

Continued feeding of thyroid to dogs leads to a 25 per cent increase in the oxygen consumption of tissues isolated from these animals. This was determined for slices of liver, auricle and vagus nerve.

The oxygen consumption of thyroid slices from the fed animals, on the contrary, is decreased 30 per cent.

The bearing of these observations on the questions of the normal control of thyroid secretion and of the action of the thyroid hormone is discussed.

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STUDIES ON THE PHYSIOLOGY OF SECRETIN

V. The Effects on the Respiration of the Excised Pancreas

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Two years after the discovery of pancreatic secretin by Bayliss and Starling, Barcroft and Starling (1904) reported a study on the effect of secretin on the gaseous exchange of the pancreas, in vivo. They observed an increased utilization of oxygen by the pancreas following an intravenous injection of their crude secretin preparation. The increased respiration was not proportional to the flow of pancreatic juice, nor did they find any direct relationship to the extent of the blood supply.

We were interested in determining the effect of highly purified secretin on the oxygen consumption by various isolated tissues.

Methods. The secretin preparation used in our experiments was our number 413 (Still, McBean and Ries, 1931). It was of the greatest potency that we have been able to prepare and had been shown to be without effect upon the vasomotor system even in amounts equivalent to 200 maximal secretory doses.

Respiration was determined in small Warburg vessels, shaken about a vertical axis in a bath at 37°C. (Gerard, 1931). Tissue slices, of sufficient thinness for oxygen diffusion, were prepared as usual, from the exposed organs in lightly anesthetized animals. Rat pancreas and diaphragm were simply excised, the former being distributed in thin, irregular clumps in the upper mesentery. After weighing on a torsion balance, tissues were placed in manometer cups in mammalian Ringer plus one-tenth volume of phosphate buffer at pH 6.9. Secretin dissolved in the same solution was placed in the side arm, alkali in the inset, and the whole flushed with oxygen.

Results. Six experiments on rat tissues have given consistent results. In each case the respiration of the pancreas rose following addition of secretin, while that of brain, diaphragm, kidney and liver (with one exception in the last) regularly continued to fall. Values of the increase of pancreas respiration, in per cent of the previous rate, for the individual experiments were: 20, 20, 25, 30, 35, 50; mean, 30. Table 1 reproduces one full experiment.

DISCUSSION. Stimulation resulting from 0.02 mgm. secretin was practically as great as from 0.2 mgm. secretin. This observation reminds one of the similarity seen in secretion experiments with the intact pancreas. By increasing the amount of injected secretin above the threshold, one soon reaches a maximum rate of response. Further increases cause a prolongation of the response but are without effect upon the rate. In each experiment, the respiratory rate of the pancreas was increased after secretin addition. The respiratory rate of the liver tissue was increased in one instance only. The other tissues studied, brain, striated muscle, kidney, were not influenced by the addition of secretin. It would appear that the stimulating effect of secretin upon the respiration of isolated tissues was specific for the pancreas. The one case when the effect was observed with

TABLE 1

4/20/31. Rat tissues in phosphate-Ringer, pH 6.9, 37°C.

TIME (PREPARED AT 11:30)	CMM. OXYGEN CONSUMED PER HOUR PER GRAM OF FRESH TISSUE			
	Pancreas	Liver	Brain	Diaphragm
12:00–12:05	710	1010	1210	580
12:05–12:15	680	900	1080	530
12:20–12:25	660	815	1110	540
12:25–12:30	630	760	960	530
Tip 0.1 mgm. secretin (12:31–12:33)				
12:37–12:42	810	815	900	540
12:42–12:52	830	790	960	540
1:02– 1:12	730	760	610	540
1:12– 1:22	730	650	480	510
—				
4:30– 4:50	510	530		490

liver suggests that this tissue may also be affected, but probably requires larger dosage. This would conform to previous observations on the liver in vivo, for it has been shown that secretin stimulates the secretion of bile by the liver but that a larger dose is necessary than that needed to cause a copious flow of pancreatic juice.

It is of interest that the stimulation of the pancreas persists for about two hours. On intravenous injection of secretin, the augmented flow usually lasts from 20 to 30 minutes. In the present cases, of course, there is no opportunity for the absorption and removal of the secretin by other tissues. A second addition of secretin, one hour after the first, was without effect, the oxygen consumption increase following the first addition being still maximal at the time of the second. Two experiments on dog pancreas gave negative results.

These experiments give further proof of the specificity of secretin and strongly suggest that the stimulus is a direct one to the secretory cells of the pancreas.

CONCLUSION

The addition of 0.02 to 0.2 mgm. of highly purified secretin to slices of rat pancreas causes an increase in respiration of from 20 to 50 per cent. Other tissues, with the possible exception of liver, are not affected.

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THE EXTRA RESPIRATION OF PHAGOCYTOSIS

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As other cells increase their energy turnover during activity, it might be anticipated that the respiration of leucocytes would increase during active phagocytic ingestion—unless the entire process is controlled by physical tensions.

Experiments were performed, using Warburg manometers under conditions described elsewhere (Gerard and McIntyre, 1932). Seven-tenths

TABLE 1

Summary of eight pairs of experiments

TIME IN MINUTES	OXYGEN CONSUMPTION OF LEUCOCYTES, IN PER CENT INITIAL VALUE	
60	100	100
After tipping	Saline	Sarcina in saline
15	97	198
55	91	130
85	94	104
145	75	80

TABLE 2

April 14, 1932. Two-tenths cubic centimeter dog leucocyte paste in 0.6 cc. dog serum; 0.1 cc. N/10 NaOH in inset; onset, 0.2 cc. Ringer, plus $\frac{1}{10}$ ink or sarcina suspension.

TIME IN MINUTES	OXYGEN CONSUMPTION OF LEUCOCYTES, IN PER CENT INITIAL VALUE		
70	100	100	100
After tipping	India ink	Sarcina in saline	Saline
15	93	423	154
45	95	128	104
105	100	81	88
165	75	78	98

cubic centimeter of a moderately thick suspension in serum of dog leucocytes, obtained by centrifuging blood, was placed in the manometer cup and, after a control period, 0.2 cc. of Ringer or a Ringer suspension (dilute) of Sarcina lutea tipped in. Manometers were gently shaken in a bath at 38°C.

Eight pairs of experiments showed a consistent increase in oxygen consumption after tipping the cocci, variable changes in the Ringer controls. The rise began at once, reached a maximum value twice that at the start in about 15 minutes, and was over in 90 to 150 minutes. Smears showed that the leucocytes were well loaded with organisms. The data are summarized in table 1 and a single protocol is reproduced in table 2.

It will be noted that the main burst of extra respiration lasts for 10 to 15 minutes, the maintained level being relatively little above the control, in individual cases below it. These may represent, respectively, the excess energy liberation of engulfment and digestion. Experiments with diluted india ink yielded negative results, the material appearing in several instances to be distinctly injurious.

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CONDUCTED CONTRACTURES WITHOUT ACTION POTENTIALS IN SINGLE MUSCLE FIBERS

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In a previous paper (1932) we reported that the submaximal responses of single muscle fibers from the retrolingual membrane were not accompanied by action potentials. The latter could only be obtained when the fiber was stimulated to respond maximally, that is, when the impulse was completely conducted. With microelectrodes, and within a certain range of stimulus strength, direct stimulation of the contractile process takes place without the appearance of the normal propagated impulse. The threshold for the latter corresponds to the strength at which Gelfan and Gerard (1930) found that graded responses abruptly changed to complete maximal twitches. If the action potential is the criterion of conduction, the submaximal responses are not conducted, and it is possible, as Gelfan and Gerard (1930) have suggested, that a localised stimulus subthreshold for conducted responses can directly stimulate the contractile mechanism.

A muscle fiber may be made to shorten by pricking or scratching the surface (Gelfan and Gerard, l.c. footnote, p. 413). The membrane, supposed to be the seat of the propagated impulse, must here be injured, and the analogy would seem to be close to the procedure of scratching the surface of the iron wire model described by Lillie. To the first prick stimulus the muscle fibers as a rule respond "maximally,"[1] but occasionally a submaximal response may be obtained. Although submaximal responses produced by electrical stimulation do not show action potentials, we naturally supposed that the complete and apparently conducted responses produced by pricking would do so, and we set out to find whether there would be the same difference between non-conducted and fully conducted responses to pricking as was found between the two types of responses to electrical stimuli.

The retrolingual membrane of the frog was excised and mounted in a moist chamber after the manner already described (Gelfan, 1930). The cathode ray oscillograph was employed for the detection of potentials.

[1] We call it "maximally," since visually under the microscope, the shortening of the fiber is as complete as may be produced in a maximal tetanus.

Relatively large silver and pore microelectrodes were used as leads from the stimulated fiber. A quartz needle was employed for scratching or pricking the fiber. Needles and electrodes were moved by micromanipulators, and observations of mechanical responses were made under the microscope. The two lead electrodes were in general placed directly on the fibers and the fibers were usually pricked near one electrode.

For recording the responses, the oscillograph beam was caused to sweep across the field at one-eighth second intervals, each line so produced, about 60 mm. long, representing 125 sigma of time. Between each excursion of the beam 3 or 4 sigma were lost in the return of the spot to its starting place. From this line as a base, deflections caused by potentials from the electrodes occurred, and all parts of the line could easily be included in the field of vision It was not practical to photograph these records because of the uncertainty as to the precise time at which a needle prick stmulus would be effective, and since in general only the form and amplitude of the response was desired, or the presence or absence of an action potential was to be noted, it was not thought worth while to take long continuous records most of which would be occupied by no significant response. Amplitudes could be approximated by comparison with a pair of dividers held against the oscillograph surface.

It will make what follows clearer to point out at once that the response evoked by a needle prick is generally neither a twitch nor a tetanus, but is in most instances a maximal contracture. Only occasionally is a twitch produced, and then apparently by scratching a nerve fiber innervating the muscle. Visually in the microscope, maximal single twitches, and contractures as produced by pricking, are so distinct as to preclude any possibility of confusion, and tetani can be recognized by series of action potentials. The first description of this phenomenon (Gelfan and Gerard, 1930) stated that these responses appeared to be tetani; the similarity to a tetanus may be rather close, but we believe that the lack of a conducted action potential is final evidence against their identity. A strong tetanus was produced in one case by scratching a nerve fiber with the needle, whereupon muscle action potentials appeared on the oscillograph at high frequency for the duration of the shortening; in the same preparation, and under identical conditions, except that the muscle was pricked instead of the nerve, no action potentials appeared. In one case, a single action potential appeared on pricking a fiber, and the mechanical response consisted of a quick twitch, followed by a longer and more pronounced shortening that could readily be distinguished from the twitch. No discontinuity is visible however in the usual contracture obtained, but the fiber shortens quite rapidly (although possibly less rapidly than in a twitch) over its whole length, remains as much shortened as by a tetanus for a second or more, and relaxes much more slowly than it contracts.

In such contractures from over one hundred and fifty fibers successfully stimulated, we were unable to detect any characteristic action potentials following a prick stimulus. The first mechanical stimulus almost invariably sends the fiber into a maximal contracture. Smaller contractures can however be easily produced by subsequent scratches of the same fiber. In neither case were characteristic muscle action potentials observed. The contractures were, to be sure, accompanied by deflections of the oscillograph, but similar deflections followed slight movements of the electrodes, or passive stretching of the muscle fiber under the electrodes. Such potential changes might be caused by sudden change of resistance between electrodes, or by mechanical disturbances of the electrode surface. The former possibility was checked by balancing out the difference of potential with a potentiometer, when a change of resistance should no longer be effective since there would be no current flowing through the electrodes. The latter possibility was checked by using pore electrodes, the metal electrode surface then being at a distance from the muscle contact where it could not be disturbed. Further, some preparations showed no detectable potential with maximal responses, and in these preparations movement of the electrodes also caused no deflection of the oscillograph. Even in the same preparation, different areas showed presence and absence of these potentials. We have finally concluded that such potentials are due to the movement past the electrodes of a field of tissue which is itself the seat of a potential gradient; a gradient of two millivolts between electrodes, or about a millivolt per millimeter, would probably account for the larger values of potential observed (a few hundredths of a millivolt). When a potential was obtained upon shortening of a fiber, passive movement of the tissue past the electrode most affected by the response, and in a direction to duplicate the relative displacement of the electrode with respect to the tissue, gave a potential in the same direction.

In the few instances when twitches occurred (recognized visually as well as by their action potentials) it was usually found that the needle touched a nerve fiber. More than one muscle fiber might then respond, and the typical action potentials observed, always diphasic, varied in amplitude depending on the distance of the fiber responding from the electrodes. When directly beneath the electrodes, each phase of such potentials was 30 to 35 mm. high at an amplification of 180 mm. per mv. Because of the short distance between electrodes (2 mm.) the spike was not only very sharp, but of very short duration (1 to 2 sigma), and the amplitude was presumably not that of a completely developed or monophasic potential. The contracture potentials, however, that could be duplicated by movement of the electrodes, lasted from twenty to forty or more sigma, and were usually monophasic and smooth, having no spike at the start. They varied from 2 to 15 mm. in height at 180 mm./mv., 1 mm. being

taken as the limit of deflection that could be read significantly, and in some experiments at a sensitivity of 380 mm. per mv., no potentials were over the latter figure. There was always complete agreement between observations of twitches in the microscope and true action potentials on the oscillograph; the slow potentials were not always present during contractures, even with the higher amplification; the latter could be either positive or negative in sign; for these reasons we conclude that they are not direct electrical signs of the activity of the contractile process, but are the indirect effects on the electrodes of the mechanical distortion of the tissue underlying them due to shortening of the muscle. We can say therefore that the contractures resulting from pricking show no electrical sign as great as 5 per cent, in some cases not over 3 per cent, of the amplitude of action potentials.

Discussion. At higher sensitivity than that specified, vibrations of the preparation in manipulation caused disturbances that masked significant observations on the oscillograph, and the values given for the potentials are only the limits we can set to any electrical sign of the contracture. Such potential signs may be not over 3 per cent of the action potentials; but even 3 per cent of a muscle action potential is not vanishingly small, although it appears small in these preparations where the single fiber is shunted by a relatively large mass of inert tissue and fluid. In sartorius preparations Bishop and Gilson (1927) report that the spike of the potential is followed by a sloping plateau that lasts throughout the contraction phase; this was thought to be an electrical sign of the contractile process, and its amplitude relative to the action potential spike was higher than the limiting figure we give here. However in these single fibers the leads are diphasic ones, and in the case quoted, monophasic potentials were dealt with. Since the contractures of single fibers are obviously conducted, the amplitude of any potential detected would depend on the activity under one electrode becoming of observable value before this was counterbalanced by a similar activity under the other electrode, and we do not know how fast the propagation was from one electrode to the other. It is possible that this is fairly fast, and that monophasic potentials if obtainable would show a higher value.

The more significant finding seems to us to be that contractures that lack typical action potentials, show conduction at all. In this case there can be no question of the spread of the stimulating agent, a less than ten micra needle prick, as there might be in the case of electrical stimuli.

In our previous paper we suggested the possibility that the non-conducted responses to electrical micro-stimulation were contractures, only because of the lack of a normal action potential. While it is perfectly obvious from visual observation that they are not conducted along the entire fiber, the submaximal responses are not only produced by single

induction shocks and tetanizing currents, but even visually it is not possible to differentiate them by their time relationships from the normal all-or-none responses. In the present case, so complete and so prompt is the shortening, that again visual observation would not certainly distinguish the contractures from tetani, and again the presence or absence of action potentials of chracteristic form is the criterion relied upon. One further difference exists, however; a tetanus can be repeated promptly by a stimulus similar to that which produces a first tetanus, and even nonconducted responses to electrical stimulation can be repeated at short intervals, but after the response to a prick, the fiber recovers its excitability very slowly, and sometimes not at all, and for a long time usually gives a lower response to a second prick. This circumstance, together with the fact that the first response to pricking is always a very violent one, almost to be described as all-or-none in character, although other contractures such as those produced by chemicals vary in intensity with the concentration of the exciting agent, indicates that the disturbance of the muscle mechanism produced by pricking is a very profound one. The conduction involved may be more of the nature of the spread of injury than the propagation of a specific impulse, although in the final analysis it would be difficult to make a distinction that was not an arbitrary one, since after all the fiber does recover to respond again.

Two questions then arise; first, why does a stimulus adequate for contractile response fail to initiate an action potential; and second, what sort of a mechanism is involved in the spreading of the response to the whole fiber, in a case where the action current mechanism, such as is found in nerve or presumably in the Lillie model is not operative.

Whereas chemicals that produce contracture can be assumed to fail to stimulate the conductile mechanism, the needle prick, that sends the fiber into a contracture, unavoidably must first pierce the membrane. One would indeed expect an action potential after puncturing the membrane.

When the muscle fiber is activated by piercing the membrane, it must be remembered that the surface area stimulated by a needle that is less than 10 mμ. in diameter is considerably smaller than the surface area of the motor end plate. The importance of the electrode size for the successful production of submaximal responses, electrically, has already been pointed out (Gelfan, 1930, 1931; Gelfan and Gerard, 1930). It seems that a stimulating agent sufficiently localized, although adequate to stimulate the contractile process, is not adequate to stimulate the transmissive mechanism as represented by the action current. In the case of microelectrode stimulation, the strength of the stimulus is regulated by the intensity of the stimulating current. But by increasing the latter, the area of high density current is also unavoidably increased. This is equivalent to increasing the electrode area. The gradation of the submaximal responses

with microelectrode stimulation is therefore limited, since the stimulus can not be maintained localized as the intensity is increased. The needle prick, however, must constitute an intense form of stimulation in order to produce a response of the entire fiber. In spite of the fact that the entire fiber is activated, the action potential is not elicited, probably because the too localized surface activation was inadequate to initiate the transmissive response. Granting the separation of the conductile and contractile mechanisms, the above explanation is not inconsistent with the iron wire model.

The action current mechanism as it occurs in nerve and muscle is certainly a highly specialized one, but it would be natural to suppose that it represented merely a specialized form of something that every cell would be found to possess. It appears at first glance to correspond to that aspect of cell function involved in irritability; but since after all the responses of cells are chemical ones yielding energy, irritation or excitation involves essentially only the release of a reaction that must be ready to proceed, and any disturbance if strong enough might effect such a release without the intervention of an action current. What the action current mechanism adds to this is rapid conduction; the process involved in this conduction can obviously set off other underlying cell processes elsewhere than at the locus of stimulation, and do it very promptly. The functions of this process might be two at least; to cause the cell to respond as a whole, and rapidly, and also possibly to serve as a sort of specialized "sense organ" that would increase the irritability of the whole cell system over that of the underlying reactions.

Now the skeletal muscle cell, being specialized to contract, might employ the action potential process to enable it to contract promptly throughout its length, even though it did not need anything to make it more irritable. As has been shown in this and the preceding paper, the muscle contractile mechanism under certain conditions of stimulation at least may be more irritable toward either electrical or mechanical stimuli than the action current mechanism itself. In the present experiments it appears to be of itself *conductile*, though perhaps not so efficiently so as is the other process. The primary contribution of the latter would therefore seem to be speed and constancy of action of the whole process, rather than increase of sensitivity. The fact that behind this action current process the muscle is still potentially conductile further suggests that the specialized conductile system responsible for the action current is not merely a specialization of the fundamental conductile potentiality of the cell, but is rather a specialized device added to this or superimposed upon it to render the whole more effective in the function it has to perform. The fact that conduction can take place without an action current, in a muscle where the action current mechanism is still intact and normally functional, indicates

a greater degree of independence of these two systems than would ordinarily be inferred from the parallel course of action potential and tension developed in many experimental procedures previously reported.

SUMMARY

Conducted contractures produced by piercing the membrane of single muscle fibers are not accompanied by action potentials.

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AN EXAMINATION OF CEREBROSPINAL FLUID FOR OXYTOCIC ACTIVITY AS TESTED BY THE RABBIT UTERINE FISTULA PREPARATION

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With the possible exception of the Guggenheim (1914) test there is at present no chemical method of detecting the presence of secretions of the posterior lobe of the pituitary gland. Investigators have, therefore, utilized the known physiological actions of extracts of this gland in testing for its secretions in body fluids. Pressor, diuretic-antidiuretic, melanophore expanding, and oxytocic properties of cerebrospinal fluid have all been reported and assumed to be due to the secretion of the posterior pituitary directly into the fluid (Cow, 1915; Cushing and Goetsch, 1910; Jacobson, 1920; Dixon, 1923; Houssay and Ungar, 1924; Trendelenburg, 1924; Jánossy and Horváth, 1925; Miura, 1925; Trendelenburg, 1926; Mestrezat and Van Caulaert, 1926; Ehrhardt, 1927; Van Dyke and Kraft, 1927; Hoff and Wermer, 1928; McLean, 1928; and Sato, 1928, among others).

In spite of the work of Van Dyke (1926) indicating that the melanophore factor is more concentrated in the pars intermedia than in the pars posterior, it has thus far been found impossible to separate the pressor, renal, and melanophore factors. However, the presence of any or all of these in C. S. F. does not prove a similar path of secretion for the oxytocic factor since the latter has been shown to be chemically separable from them (Kamm, et al., 1928).

C. S. F. from humans and dogs has been tested on isolated strips of guinea pig or mouse uterus and reported to contain an oxytocic principle by the workers listed above. The results of those who gave definite values for the concentrations of the substance are shown in table 1. It has also been reported by some investigators (Cow, 1915; Dixon, 1923; Dixon and Marshall, 1924; Blau and Hancher, 1926; Siegert, 1927; Hoff and Wermer, 1928a; Karplus and Peczenik, 1930) that after the "hypophyseal secre-

[1] Presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Pennsylvania.

[2] This investigation was aided by a grant from the Committee on Research in Problems of Sex of the National Research Council.

tion" had been stimulated by various physiological or artificial procedures, the oxytocic factor was found in increased concentrations in the C. S. F. (or that it could then be detected in fluid where the method used did not indicate its presence normally). Although McLean (1928) obtained oxytocic values for C. S. F., they were lower than those he found for venous blood, and he, therefore, felt that the route of secretion is into the blood stream, the material being absorbed from there into the C. S. F.

Recently, however, Van Dyke, Bailey and Bucy (1929) have shown that increases of the $[Ca^{++}]$ of the bath surrounding an isolated uterine strip may evoke a response which exactly simulates the addition of pituitrin. They, therefore, believe that the oxytocic activity reported in C. S. F. by other workers is due solely to the fact that the $[Ca^{++}]$ of the fluid was higher than that of the bath to which it was added.

TABLE 1

Concentration of oxytocic substance in C. S. F. reported by previous workers

The values in this table are calculated on the assumption that 3 mgm. fresh gland = 1 international unit and that "Pituitrin" contains 10 international units per cubic centimeter.

NAME	REFERENCE	TYPE FLUID	INTERNATIONAL UNITS PER CUBIC CENTIMETER
Dixon	1923	Dog cisternal	0.02–0.2
Trendelenburg	1924	Cat cisternal	0.0001 average
Miura	1925	Cat cisternal and lumbar	0.00013 average
Van Dyke and Kraft	1927	Dog cisternal	0.00007–0.0007
		Human lumbar	0.0004 average
McLean	1928	Dog cisternal	0.000002–0.00024 0.00002–0.00003 usually

The development of the rabbit uterine fistula technique (Reynolds, 1930) offered a way to escape the difficulties inherent in isolated tissue experiments. By injecting intravenously, the buffers of the rabbits' blood are utilized to prevent radical ionic changes in the uterus and the solutions do not have to be accurately buffered or neutralized.

Previous work on the preparation has shown (Weinstein, Reynolds and Friedman, 1931) that the injection of foreign proteins evokes no response in the uterus unless the rabbit has been previously sensitized to the protein used. In other experiments (Weinstein and Friedman, unpublished) no uterine reaction has been obtained when as much as 8 cc. calcium gluconate (= 80 mgm. Ca) have been injected at one time. The fistula does not respond to injections of 0.2–0.6 mgm. of histamine although 0.5–1.3 mgm. per kilo body weight is the lethal dose in the rabbit.

The fistula thus affords a uterine preparation which is relatively, at least,

insensitive to changes of ionic concentration (including Ca ions) or the addition of protein or histamine. With this rather specific test object the question of the presence of the posterior pituitary oxytocic factor in the C. S. F. of dogs and humans has been reinvestigated.

METHODS. 1. *Animals.* The rabbits used were all post partum animals as these have been shown (Reynolds, 1931) to have better uterine activity and to be more sensitive to pituitrin.

2. *Uterine fistulae* were made from 1 to 18 days post partum (usually within 5 days) with the technique and precautions described by Reynolds (1930) and Reynolds and Friedman (1930). The animals were first used for recording not less than three or four days after operation.

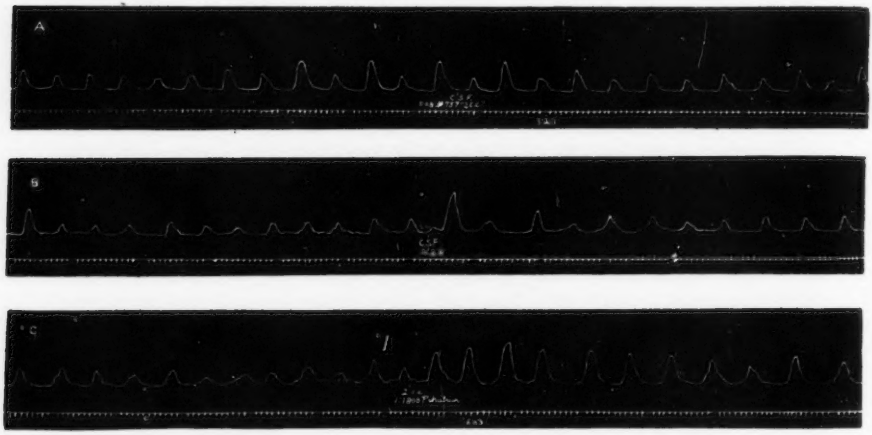

Fig. 1. A. Negative result of injection of 2 cc. dog C. S. F.

B. Negative result of injection of extract of human C. S. F. representing original volume of 92 cc.

C. Result of later injection in same experiment as A and B of 2 cc. 1:1800 pituitrin. Response shows less effect on tonus and more on size of contraction than usual response to small doses.

Time: 5 seconds. One-fourth original size.

3. *Experiments* were recorded as described by the above workers. The sensitivity of the rabbit was checked before and after the injection of fluids by the administration of known amounts of pituitrin. All injections were made intravenously at intervals of not less than ½ hour.

4. The *standard* of comparison was "Pituitrin" or "Pitocin."[3] Each of these preparations contained ten international oxytocic units per cubic centimeter. The potency of two bottles of "Pituitrin" was checked in our laboratory against a solution of standard pituitary powder from the U. S. Hygienic Laboratory and found to

[3] These extracts were very generously furnished through the courtesy of Dr. Oliver Kamm and Dr. E. P. Bugbee, by the research department of Parke, Davis & Co.

contain ten international units per cubic centimeter within the limits of accuracy of our method of assay. All dilutions were made just before using, in 0.9 per cent NaCl solution.

5. *Cerebrospinal fluids.* a. *Dog.* The fluid was collected by cisternal puncture from dogs anesthetized with ether after subcutaneous morphine and atropine. The fluid used was usually macroscopically clear (see table 2) but a control showed (see below) that bloody fluid could also be used.

b. *Human.* Human fluid was obtained[4] by lumbar puncture (local anesthesia) from both male and female psychopathic patients (maniacs, paretics, alcoholics, etc.).

TABLE 2

Experiments with dog cerebrospinal fluid

DOG NUMBER	TIME C. S. F. KEPT	DESCRIPTION OF C. S. F.	C. S. F. INJECTED (NO RESPONSE)	RESPONSE TO PITUITRIN INJECTIONS OF	THEREFORE C. S. F. MUST CONTAIN < INTERNATIONAL UNITS PER CUBIC CENTIMETER
			cc.		
1	13 days	Bloody	1.0	1.0 cc. 1:1000	0.01
1	3 days	Clear	2.0	2.2 cc. 1:500	0.022
2	3 days	Clear	2.0	2.2 cc. 1:500	0.022
3	7 days	Clear	2.9	3.2 cc. 1:800	0.014
4	13 days	Clear	2.0	2.0 cc. 1:1800	0.0055
5	6 days	Somewhat bloody	2.0	2.0 cc. 1:500	0.02
6*	6 days	Clear	2.0	2.0 cc. 1:500	0.02
7*	1 day	Clear	2.0	2.0 cc. 1:500	0.02
8*	2 days	Clear	2.0	2.0 cc. 1:500	0.02
3	2 hours	Clear	2.0	2.0 cc. 1:1000	0.01
4		Tinged			
6		Clear			
9		Tinged			
7	6 hours	Clear	2.0	2.0 cc. 1:500	0.02
10		Clear			
8		Clear			
11		Clear			

* These samples are also included in the composite fluids.

The fluid (dog or human) was collected into tubes packed in ice, was immediately acidified to approximately 0.25 per cent with acetic acid, and was kept on ice until used. Just before injection the fluid (or extract) was roughly neutralized with $NaHCO_3$ in order to prevent irritation of the rabbits' veins.

RESULTS. The enormous difference between the values reported by previous workers for the oxytocic material in C. S. F. is obvious from table 1. It has been possible to cover the highest figures (given by Dixon)

[4] For the large amounts of human fluid required we are greatly indebted to the Staff of the Psychopathic Department of the Philadelphia General Hospital without whose coöperation this part of the work would have been impossible.

using unconcentrated dog fluid. As this has, at times, taxed the sensitivity of the method, experiments have also been undertaken in which large amounts of human fluid have been concentrated and extracted before injection.

A. *Unconcentrated dog cerebrospinal fluid.* The results with unconcentrated dog fluid are summarized in table 2. It will be noted that in each case the C. S. F. had no effect although the uterus responded to doses of pituitrin equal to or less than that which should have been contained in the fluid if the minimal amount reported by Dixon were present.

In two cases the fluids injected were each composed of equal parts of samples from four dogs. The first of these (from animals 3, 4, 6 and 9) indicates that the average for the four fluids is less than *one-half* of Dixon's minimum. Since one of the four was also shown separately to be below the minimum, this is strong evidence that all were.

In the second group of four fluids (animals 7, 10, 8 and 11) the lack of response to the C. S. F. indicates that the average content of oxytocic material must be less than Dixon's minimum. Two of the samples were also shown individually to be below it. Under these circumstances it seems improbable that the other two were above it.

It may be questioned whether pituitary hormone, even if originally present, is stable in the fluids for the periods which they were kept (2 hours to 13 days). Therefore, to four different *composite* fluids, one of which was somewhat bloody, we added sufficient pituitrin to make its composition in the range of Dixon's maximal value (for greater ease in titrating). These were then compared, after varying intervals, to fresh pituitrin solutions. The difficulties in titrating these controls and the validity of the results thus obtained are fully taken up in the discussion. It may be stated here that in the control fluids kept 6, 9, 28 and 28 days any deterioration, if present, must have been less than 25 per cent. However, it must be emphasized that there was no real evidence of any loss. As one of the controls kept the longest time was macroscopically bloody, the use of somewhat bloody fluid experimentally seems justifiable.

B. *Concentrated human fluid.* Composite samples of human C. S. F. totaling 36 cc. to 92 cc. each were dried in evaporating dishes over a hot plate with an electric fan. The residue was extracted with four or five washings of 95 per cent ethyl alcohol and centrifuged to remove the salt. The decanted liquid was again dried as above and the residue extracted with 0.25 per cent acetic acid made in 0.9 per cent NaCl. Four or five washings were used, and the final volume was 1.5 to 2 cc.

A series of thirteen known pituitrin solutions made in 0.9 per cent NaCl (25 cc. amounts) was extracted by this method and the end products titrated against fresh pituitrin solutions. In eight cases the yield was 80 per cent or over; in two it was less than 65 per cent (one about 60 per cent

and one still lower); and in the remainder it was less than 75 per cent. The mode was around 80 per cent. (It is probable that the error in such titrations is at least 10 per cent in either direction. However, in view of the usual loss of sensitivity of the preparation during the experiments they were consistently conducted in such a manner that the chances for high estimations of yields were minimized while the chances for low estimations were increased.)

An at present incomplete group of three control fluids has been made by adding pituitrin to human C. S. F. (composite samples totaling 15 to 65 cc.). The original concentration in two cases approximated the maximum reported by Van Dyke and Kraft (1927), while the third had a somewhat greater pituitrin concentration. These fluids were then extracted by the method just described and the yields determined. In one case the yield was estimated as greater than 65 per cent and less than 80 per cent. A

TABLE 3

Experiments with extracts of human cerebrospinal fluid

EXTRACT NUMBER	ORIGINAL VOLUME OF C. S. F.	RESPONSE TO PITUITRIN INJECTIONS OF	THEREFORE C. S. F. MUST CONTAIN < INTERNATIONAL UNITS PER CUBIC CENTIMETER*
	cc.		
1	36	2 cc. 1:870	0.0009
2	65	1.6 cc. 1:800	0.00044
3	87	1.8 cc. 1:750	0.00038
4	90	1.9 cc. 1:750	0.00038
5	58	2 cc. 1:1800	0.00027
6	92	2 cc. 1:1800	0.00017

* Allowing for 30 per cent loss as discussed in the text.

month later on another rabbit the same extract was titrated as between 80 per cent and 85 per cent. The results from the other two extracts in this series also showed yields around 80 per cent. Since these results are in close agreement with those on the saline solutions, we feel justified in including the latter as further evidence that our method of extraction will recover in most cases 70 per cent to 80 per cent of pituitrin added to C. S. F.

In table 3 are summarized the results of the injections of extracts of C. S. F. to which no pituitrin had been added. No response was ever obtained from any fluid although in each case the whole extract was injected at one time. Yet the pituitrin responses in these experiments indicate that in five of the six cases the concentration of oxytocic substance, if present, must have been *less than* the *average* reported by Van Dyke and Kraft even if it is assumed that a loss of 30 per cent occurred during extraction. As each extract represented fluid from at least ten patients, each of these

values is comparable to the *average* values of previous workers. (Strictly speaking, they are not true averages as the amounts of fluid from different patients varied greatly.)

Discussion. The apparently nearly specific character of this method for detecting pituitrin-like material outweighs, we believe, any disadvantages in its use for problems such as that undertaken in the present investigation. Practical disadvantages are, however, present, and it is impossible to evaluate the experimental or the control data without considering the experimental difficulties encountered.

The principal disadvantages of the method lie in the low and variable sensitivity. At best the fistula is much less sensitive than the isolated strip. (In a few instances only was a response to 0.004 international unit obtained although responses to 0.008 international unit were more frequently seen.) In addition to this, enormous variations in sensitivity were seen from rabbit to rabbit. At present we can not definitely explain these differences. Moreover, it was found that the threshold for the same rabbit also fluctuated. There was usually a progressive decline in sensitivity in the days following the first use of the preparation (good fistulae were used up to 10 to 14 days after the operation).

In addition to these difficulties, there was invariably a decrease in response to pituitrin in the course of each experiment. This occurred in spite of the precaution of allowing a half hour to elapse between injections.[5] The rate of the loss of sensitivity was not a constant one even on the same day (it was liable to be much more rapid after the animal had been in use for several hours than at first). In the present experiments the sensitivity to pituitrin was, therefore, checked after each C. S. F. or control fluid injection.

In these experiments we also had two possible sources of error which are not inherent in the method but were due to the fact that we were using small, almost minimal doses of pituitrin.

The first of these is one which might easily lead to false results unless precautions are taken to avoid it. It was found that not only the amount of pituitrin injected but also the concentration influenced the response; an animal which responded to 0.4 cc. of 1:400 pituitrin did not respond to 1.6 cc. of a 1:1600 dilution although the amount of pituitrin injected was obviously the same. (This dilution effect is greater in some animals than in

[5] Other work in this laboratory (Weinstein and Friedman, to be published) has indicated that the uterine refractory period after an injection of pituitrin (reported by Frankl-Hochwart and Fröhlich, 1910; and Fuhner, 1913) lasts approximately twenty minutes after intravenous injections of the magnitude used in the present work. However, pituitrin was often injected ten minutes after an injection of C. S. F. and no change in the response was seen which could have been attributed to a tolerance due to the preceding fluid.

others and can be somewhat reduced by the use of larger hypodermic needles which allow a more rapid injection.) In the experiments reported in this paper, therefore, the volumes of the corresponding control pituitrin injections were always made equal to or greater than those of C. S. F. given. (Using these larger, more dilute injections our most sensitive preparation responded well to 0.1 international unit—1 cc. of 1:1000 pituitrin.)

The second special difficulty due to the small doses of pituitrin injected was that in some preparations spontaneous motility changes occurred which simulated the pituitrin responses obtained in these rabbits. In most of these the experiment was discarded. However, in the case of three dog fluids it was readily apparent that the change, which followed the known pituitrin injection, was a pituitrin response (and not one of these spontaneous variations) since it could be obtained repeatedly, each time with the correct latency. In addition, no such effect was ever seen following an injection of C. S. F. No C. S. F. was considered negative unless a valid pituitrin response was obtained *after* the fluid was injected.

It is due to these difficulties that it has been impossible to obtain more accurate titrations of the controls for the preservation of pituitrin in dog fluid since the loss of sensitivity happened to be particularly rapid in the animals of this series. Nevertheless these experiments do clearly show that if there was any deterioration in the control C. S. F., it was not greater than 25 per cent. For example: 0.5 cc. of a 1:10 dilution of pituitrin was added to 10 cc. of a composite sample of dog C. S. F., making a pituitrin dilution of 1:200. This control C. S. F. was then kept 28 days before being titrated. The early part of the titration showed no difference between suitable amounts of this control fluid and of fresh pituitrin solutions. It was then found that the response to 0.3 cc. of a fresh 1:200 pituitrin solution was quite comparable to the next injection which was of 0.4 cc. of the control C. S. F. (made as a 1:200 dilution originally). This might have indicated a 25 per cent deterioration in the control fluid were it not for the fact that the final injection of 0.4 cc. of a fresh 1:200 pituitrin solution now gave a response only slightly, if at all, larger than that previously given by the injection of 0.3 cc. of the same solution. This showed a loss of sensitivity in the preparation. This loss might just as well have occurred between the time of the 0.3 cc. of the fresh pituitrin solution and the control fluid as between the latter and the final fresh solution injected.

When the saline solutions of pituitrin were extracted, sufficient pituitrin could be used to make the final extract relatively concentrated and thus allow more dilutions. Consequently, it was possible to obtain more accurate titrations even though the sensitivity of the fistula had decreased during the experiment. With extracts of pituitrin in human C. S. F. the entire amount, representing an initial volume of 15 to 65 cc., usually had to be injected at once in order to evoke a response. The matching of the response by a fresh pituitrin solution was therefore more difficult and more liable to error from variations in sensitivity of the preparation. There was however no evidence that the loss of pituitrin was any greater in the extracts of C. S. F. than in those of saline solutions (20 per cent to 30 per cent).

The controls thus show that pituitrin will keep in C. S. F. under our experimental conditions whether the fluid is used as obtained or is extracted. In the first series of experiments (with unconcentrated dog C. S. F.) the uterine preparations should have given a response if the experimental fluids contained Dixon's minimal concentration of oxytocic substance (or less in some cases). We, therefore, wish to advance the data in this paper as evidence that there is no pituitrin-like material in the C. S. F. of the dogs of our laboratory in amounts approaching those postulated by Dixon. As can be seen from table 1, other workers reporting the presence of an oxytocic substance in C. S. F. have all found it in a much smaller concentration than did Dixon. Blau and Hancher (1926) in an attempt to repeat Dixon's experiments were unsuccessful at least so far as normal fluid was concerned. The data obtained by our method thus confirm these previous investigators.

Whether the pituitary normally secretes into C. S. F. or whether Van Dyke, Bailey and Bucy (1929) are correct in their belief that the oxytocic effect reported by others is due to a Ca ion concentration difference, we can not at present say. Our preliminary experiments on concentrated C. S. F. showed that the amount of pituitrin in the original fluid, if present at all, must have been less than 0.0009, 0.00044, 0.00038, 0.00038, 0.00027, and 0.00017 international unit per cubic centimeter. Since the average value reported by Van Dyke and Kraft (1927) was 0.0004 international unit per cubic centimeter (see table 1) this indicated that we could find no pituitrin-like material in C. S. F. in amounts approximating those reported by these workers. Three possible explanations for this negative result occur to us.

First, the pituitary may secrete into C. S. F. but in still smaller amounts so that the concentration of the secretion more nearly approximates those reported by Miura, Trendelenburg, and McLean. We hope to enlarge our series using still larger amounts of C. S. F. in order to cover the full range of values reported by all these workers.

Secondly, it is theoretically conceivable that the true pituitary hormone differs sufficiently in chemical properties from pituitrin to lead to its destruction by the method of extraction used in this work even though pituitrin is not destroyed. We believe that this is highly improbable.[6]

[6] It should be pointed out that the human fluids used were obtained from cases in which there was no clinical evidence that the hypophysis or its path of secretion was in any way abnormal due to nearby neural lesions. This, we believe, eliminates the basis for the objection raised by Carlson and Martin (1911) to the work of Cushing and Goetsch (1910) who used patients showing hypophyseal and neural disturbances. The further objection raised by these workers—namely, that the final fluid was a highly concentrated salt solution—we have met by the alcoholic extraction, which removes practically all of the salt present.

The third possibility is that the oxytocic activity of C. S. F. previously reported was due to something other than the posterior pituitary hormone, presumably to a Ca ion effect. We regard this as the most plausible explanation of our results. We hope to be able to show this more definitely by the further work mentioned above. Attempts will also be made to increase the oxytocic factor by the injection of the various substances used by previous workers or by the use of fluid from pregnant animals and to determine if the effect is due to increased hypophyseal secretion as they believed.

SUMMARY

1. The use of the rabbit uterine fistula as a test for the presence of pituitrin is described. The advantages and disadvantages of the method are discussed.

2. Using the acidified cisternal fluid of normal dogs it has been found impossible to show the presence of a pituitrin-like oxytocic material in the amounts reported by Dixon for such fluids.

3. A method of concentrating and extracting dilute solutions of pituitrin is described.

4. Preliminary experiments on large amounts of fluid from psychopathic patients (lumbar puncture) extracted by this method have failed to reveal any oxytocic material in amounts approximating those found by Van Dyke and Kraft.

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THE CONTINUOUS INTRAVENOUS ADMINISTRATION OF EPINEPHRINE AND GLUCOSE IN DOGS: FURTHER OBSERVATIONS

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In a previous communication (1) we have described some of the chemical effects resulting from the continuous intravenous injection of epinephrine in unrestrained dogs. In order to determine the effects of injecting epinephrine and glucose simultaneously, and consecutively, a second group of experiments on dogs was performed.

EXPERIMENTAL METHODS. Jacobs (2) has previously described the apparatus for continuous injection. In the experiments in which epinephrine and glucose were injected alternately, a second injecting unit was assembled which could be attached to the intravenous cannula at once. The entire apparatus was sterilized with a 10 per cent formalin solution after each experiment. The operative technique, fluid administration and general plan of the experiments were similar to those reported previously (1).

Normal healthy medium-sized dogs were used. In brief, after a day without food an intravenous cannula was inserted into the jugular vein under local anesthesia (novocaine) and physiological saline solution was injected during a "fore-period" of from fifteen to twenty-four hours. Numerous observations have shown that the operative manipulations are sufficient to cause a transient hyperglycemia and occasionally a glycosuria, both of which have always subsided by the end of twenty-four hours. The dogs were given no food during the experiment but were allowed water as desired. The weight loss averaged about 75 grams a day.

Blood samples for sugar and cholesterol determinations were collected in dry potassium oxalate tubes from the saphenous vein and femoral artery. To facilitate the rapid drawing of venous blood, small incisions were made through the skin over the distended vein at the time of operation. The blood was analyzed for sugar content according to the Folin-Wu method

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(3) and for total cholesterol according to the method of Lieboff (4). The urine was collected in periods of from four to twenty-four hours. At the close of each period the animal was catheterized and the cage floor rinsed. Each fraction was run in duplicate for sugar according to the method of Folin and Berglund (5) and for total nitrogen by the Kjeldahl method.

Epinephrine (Mulford-Adrin 1–1000) was used. Fresh dilutions were made up every twenty-four hours. The rates of administration were from 0.001 to 0.002 mgm. per kgm. per minute (0.060 to 0.120 mgm. per kgm. per hour). Cannon and Rapport (6) have estimated that in cats the maximum rate of endogenous epinephrine secretion from splanchnic stimulation is from 0.003 to 0.005 mgm. per kgm. per minute, in accordance with which our rates of administration were within physiologic limits.

Glucose (Mallinckrodt-Dextrose reagent) was made up in 5 and 10 per cent strengths and autoclaved. In five of the experiments the glucose was dissolved in distilled water. In eight it was dissolved in 0.6 per cent sodium chloride solution. The rates of administration were from 0.3 to 0.6 gram per kgm. per hour. At these rates there is no gross glycosuria from the injection of glucose alone although there may be a temporary increase in blood sugar percentages (Sansum and Woodyatt, 7; Felsher and Woodyatt, 8).

Epinephrine was diluted with the glucose in sterile volumetric flasks and samples analysed for sugar content according to the method of Folin and Berglund. When epinephrine was administered alone it was diluted with physiologic saline solution.

EXPERIMENTAL RESULTS. Experiments were carried out on thirteen dogs and lasted for from 72 to 218 hours. In 9 of these, glucose and epinephrine were injected as components of the same solution. In 4, glucose alone was injected for varying lengths of time *following* a period of epinephrine administration. The experiments are divided into "periods" of approximately twenty-four hours each. The Roman numerals mark identical periods in the corresponding graphs and tables.

1. *Effects on blood sugar.* In each experiment the continuous administration of epinephrine and glucose caused a marked hyperglycemia, the maximum of which occurred in about four hours. There was then a gradual fall to normal (80 to 120 mgm. per 100 cc.) in from thirty-six to forty-eight hours and this normal glycemia persisted even when the rate of epinephrine administration was doubled. If the injection was stopped during this time the blood sugar values fell within an hour to low normal or slightly hypoglycemic levels; and they remained low for the next twenty-four hours or longer. On again starting epinephrine and glucose at the original rates the original hyperglycemic curve could be duplicated almost exactly.

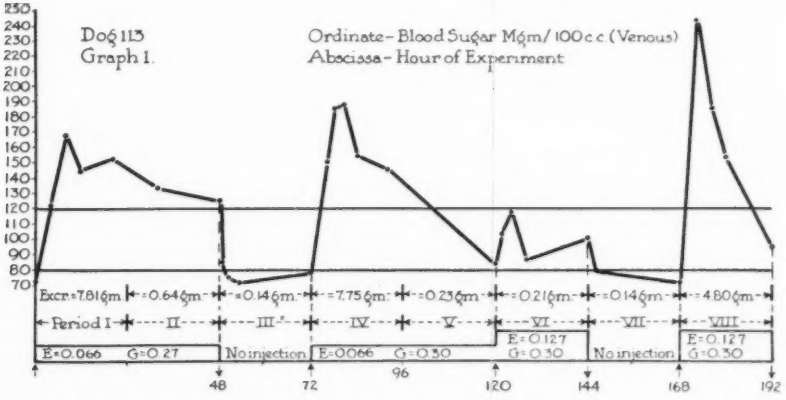
In the experiments in which glucose alone was administered immediately

following the continuous injection of epinephrine alone, there was no hypoglycemic phase on stopping the epinephrine. A transient hyperglycemia of from one to two hours duration occurred and there was an increase in the arterio-venous blood sugar differences during this time

TABLE 1

(See graph 1.) Dog 113. Weight 8.4 kgm. Physiologic saline solution: 23 hours at 50 cc. per hour. Blood sugar at end of this period 73 mgm. per 100 cc. Glucose, 5.0 per cent in 0.6 per cent saline solution.

HOUR OF EXPT.	PERIOD (24 HOURS)	INJECTION			EXCRETION		
		Epinephrine	Glucose	Glucose total	Urine for period	Urine glucose	Urine N
		mgm./kgm./hr.	*gram/kgm./hr.*	*grams*	*cc.*	*grams*	*grams*
0–24	I	0.066	0.27	54.3	1,230	7.81	3.74
24–48	II	0.066	0.27	54.9	1,560	0.64	2.13
48–72	III	No injection			730	0.14	1.90
72–96	IV	0.066	0.30	60.6	1,270	7.75	2.42
96–120	V	0.065	0.30	60.0	1,220	0.23	1.88
120–144	VI	0.127	0.29	58.2	1,300	0.21	2.48
144–168	VII	No injection			890	0.14	3.65
168–192	VIII	0.132	0.30	60.0	1,050	4.80	2.93



Graphs 1 and 2. *E.* = epinephrine administration, milligrams per kgm. per hour. *G.* = glucose administration, grams per kgm. per hour. *Excr.* = urinary glucose excretion for period.

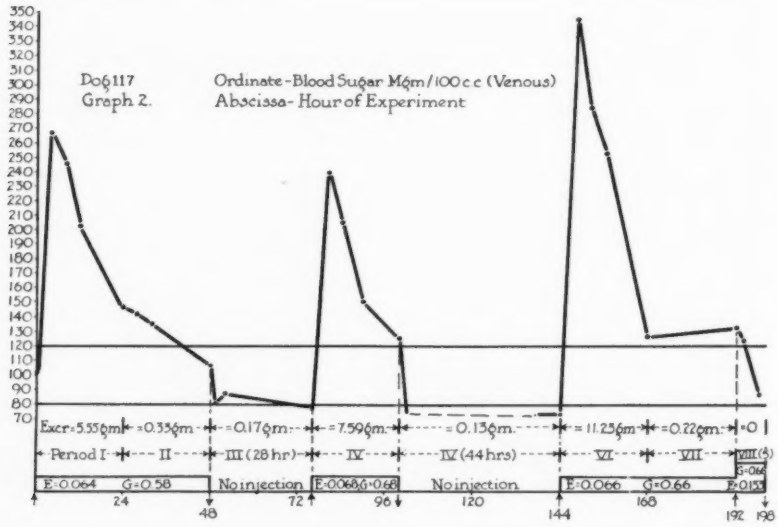
(average, 29 mgm. per 100 cc.). Thereafter the blood sugar values again fell to normal. During a second period of epinephrine injection after 24 hours of glucose administration, the original hyperglycemia could be duplicated; but this did not occur if the glucose had been injected for less than 16 hours.

2. *Effect on glycosuria.* In all experiments the administration of epinephrine and glucose led to an immediate gross glycosuria which had ceased by the end of twenty-four hours. With the exception of one dog the

TABLE 2

(See graph 2.) Dog 117. Weight 7.5 kgm. Physiologic saline solution: 17 hours at 45 cc. per hour. Blood sugar at end of this period 101 mgm. per 100 cc. Glucose, 9.1 per cent in 0.6 per cent saline solution.

HOUR OF EXPT.	PERIOD	INJECTION			EXCRETION		
		Epinephrine	Glucose	Glucose total	Urine for period	Urine glucose	Urine N
		mgm./kgm./hr.	*gram/kgm./hr.*	*grams*	*cc.*	*grams*	*grams*
0–24	I	0.064	0.58	105.2	1,140	5.55	1.65
24–48	II	0.063	0.58	104.3	1,050	0.33	1.90
48–76	III (28 hours)	No injection			320	0.17	2.18
76–100	IV	0.068	0.68	123.0	1,320	7.59	1.97
100–144	V (44 hours)	No injection			260	0.13	3.08
144–168	VI	0.065	0.65	118.6	1,160	11.23	2.09
168–192	VII	0.067	0.67	121.4	920	0.22	1.28
192–198	VIII (5 hours)	0.133	0.67	25.0	Incomplete	0(qual)	



quantitative excretion during this time varied from 5.5 to 39.4 grams (5 to 32 per cent of the intake), and of these amounts from 50 to 75 per cent was excreted during the first eight hours. The other animal excreted 105.2

grams during the first twenty-four hours (80 per cent of the intake) and the urine still reduced Haines' solution at the end of forty-eight hours. When the injection was continued for a second twenty-four hours the quantitative excretion was but little more than found under normal conditions.

If after a period without injection, epinephrine and glucose were again administered a second glycosuria resulted which equalled or exceeded the first. When glucose was administered at approximately 0.6 grams per kgm. per hour a third period of epinephrine and glucose injection led to a glycosuria nearly double the first (table 2, period VI). When glucose was administered at approximately 0.3 gram per kgm. per hour the glycosuria during a third period of epinephrine and glucose injection was less than the first even when the rate of epinephrine administration was doubled and higher blood sugar values were obtained (table 1, period VIII).

3. *Effect on total urinary nitrogen excretion.* The constant administration of epinephrine and glucose appeared to have no definite effect upon the total nitrogen excretion in the urine. At times the total nitrogen excretion during a glycosuric period was from 0.5 to 1.0 gram higher than during the following period when there was no glycosuria, but the reverse was also true. The excretion for periods without injection did not vary markedly from the average. In the longest experiment there was a gradual increase in total nitrogen excretion from 2.8 to 5.6 grams (by 24 hour periods) regardless of the experimental procedure. Table 3 shows the nitrogen

TABLE 3

Dog 121. Weight 9.5 kgm. Glucose (10.1 per cent in 0.6 per cent sodium chloride solution): 0.58 gm. per kgm. per hour. Epinephrine: 0.063 mgm. per kgm. per hour. Fluid: average 55 cc. per hour.

HOUR OF EXPT.	LENGTH OF PERIOD	URINE VOLUME	URINE GLUCOSE EXCRETION	URINE N (TOTAL) EXCRETION	
	hours	*cc.*	*grams*	*grams*	
0–24	4	500	11.71	0.64	
	4	390	8.28	0.39	
	4	400	3.48	0.50	
	4	420	0.90	0.47	
	8	640	1.78	0.82	
	Total 24	2,350	26.15	2.82	1st 24 hours of injection
24–48	Total 24	2,120	0.50	1.91	2nd 24 hours of injection
48–72	4	140	0.04	0.43	
	20	480	0.06	2.91	
	Total 24	620	0.10	3.34	no injection

excretion to be constant during periods in which there was a decreasing glycosuria.

4. *Effect on blood cholesterol.* The injection of epinephrine and glucose led to a hypercholesterolemia in the majority of experiments. In two, however, normal blood cholesterol percentages prevailed throughout. Values between 90 and 120 mgm. per 100 cc. were generally found at the end of the fore-period although one of 65 was observed. The hypercholesterolemia reached a maximum slowly, the length of time varying from twelve to fifty-two hours after the start of injection. After the maximum the blood cholesterol values tended to fall again to normal. The greatest increase above "normal" in five experiments respectively was 42, 76, 79, 79 and 82 mgm. per 100 cc.

The observations on blood cholesterol percentages were made because of a report by Kajimura (9) who injected rabbits with repeated doses of epinephrine and noted a hypercholesterolemia similar to that obtained by feeding cholesterol. He found arterial changes proportional to the hypercholesterolemia.

5. *Apparent effect of sodium chloride in the injecting fluid.* In four dogs no sodium chloride was used in the injecting fluid. In the other nine dogs a 0.6 per cent sodium chloride solution was used. Other conditions (rate of epinephrine injection, etc.) were similar in both groups. In comparing the results of the two sets of experiments it was found that in the first group the dogs did not stand the injection well. Three were moribund and the other in only fair condition at the end of the experiment, two after receiving only half as much epinephrine as the dogs in the second group. The hyperglycemia in general reached a higher level and was more prolonged. The urinary sugar excretion averaged 38.5 per cent of the glucose intake during the first twenty-four hours. In the second group the dogs were all in excellent condition at the end of the experiment. The urinary sugar excretion for a similar length of time averaged 16.4 per cent of the glucose intake.

COMMENT. A comparison of previous results (1) with those above lead to a better conception of the action of constantly administered epinephrine. It is generally agreed that one of the primary actions of epinephrine is a liberation of the available glycogen of the body and that the liver is the greatest single source of such glycogen (10).

Following Woodyatt's conception (11) it is believed that the quantitative glycosuria over a period of 24 hours is a better criterion of the amount of available glycogen in the body than is the degree of a hyperglycemia.

It is hard to see how the present experimental results can be interpreted on the basis of increased glycogen deposition in the liver following epinephrine injection such as has been reported by Drummond and Paton (12),

Pollak (13) and Markowitz (14). Ordinarily glucose injection increases the glycogen stores and the administration of epinephrine following such injection leads to a glycosuria and hyperglycemia. Under the present experimental conditions where epinephrine and glucose are given together there is no further hyperglycemia or glycosuria after the initial increase has subsided even when epinephrine is administered at double the original rate or when a single large dose (1 mgm.) of the drug is given. This would tend to show that less liver glycogen was present than at the beginning of the experiment. A period without injection is then necessary before further epinephrine administration leads to a glycosuria and hyperglycemia which parallels the original. Previous histological studies (15) have further strengthened the belief that continuous epinephrine injection decreases liver glycogen. Best's carmine stain revealed only questionable traces of glycogen in the livers of these dogs. The controls were dogs that had been starved for two weeks. Their livers showed glycogen in stainable amounts.

The obvious objections to the work of men reporting increased amounts of liver glycogen following epinephrine injection are the lack of adequate controls (12) and the failure to take the time element between injection and liver analysis into consideration (13), (14). For example, Markowitz (13) made liver analyses from 2 to 4 hours after the last dose of epinephrine had been given and at that time the blood sugar values were normal. Knowing that epinephrine is rapidly destroyed in the body and that the organism tends to replenish its glycogen reserves quickly it is entirely possible that his analyses were made after the peak of epinephrine action.

In conclusion it is suggested that by the action of constantly administered epinephrine varying amounts of glycogen are released from the animal body (the amount depending upon the state of nutrition) which are measured proportionately over a period of time by the quantitative glycosuria. Glycogenesis and glycogenolysis particularly in the liver come into an equilibrium at a much lower level than before. The storage of additional glycogen in the liver so that it may be available for epinephrine mobilization is not accomplished until *after* epinephrine injection has ceased. The deposition still cannot come up to the pre-existing level in 24 hours unless an outside source of glycogen is provided (in the present experiments, injected glucose) and further injection results in a lessened glycosuria. When sufficient glucose is injected to replenish the glycogen reserves more completely than before, further epinephrine and glucose administration results in a glycosuria and hyperglycemia greater than the original.

Such an hypothesis can further assist in the explanation of the apparent paradox of higher blood sugar values and a decreased glycosuria which occasionally accompanies the injection of epinephrine at a double rate (graph 1, period VIII, and (1)). The higher *rate* of glycogen discharge resulting from the higher rate of epinephrine injection leads to greater

blood sugar percentages; but since the amount of available glycogen is less than before the quantitative glycosuria is less.

Cori's observations (10) on the initial decrease and later increase of liver glycogen in rats following single doses of epinephrine do not necessarily conflict with our conclusions. Possibly generalized glycogen mobilization followed by a decrease in epinephrine effect from destruction of the drug leads to a piling up of glycogen in the liver similar to the temporary abnormal glycogen deposition in the kidney during the early stages of phlorhizination.

The fact that there is no great variation in the urinary total nitrogen excretion would seem to indicate that the injection of epinephrine and glucose has no marked effect on protein metabolism.

The question of the suppression of carbohydrate metabolism by epinephrine injection has been discussed by Cori (10) following the work of Colwell and Bright (16). In view of the objections raised to the use of amytal as an anesthetic in work on carbohydrate metabolism by Hines and his co-workers (17) and the observations on the apparent synergistic action of epinephrine and amytal by G. T. Cori (18), the interpretation of results in experiments in which this anesthetic is used may be open to question. The present experiments do not offer support for the assumption of a complete suppression of carbohydrate metabolism. If this were true one might expect a constant glycosuria equalling the glucose intake after an equilibrium had been established and also an increase in total nitrogen excretion from increased protein metabolism. Neither of these was observed. Only one animal showed a constant excretion (76 to 86 per cent of the intake) for six four-hour periods and the blood sugars remained elevated. This animal was given no sodium chloride in the injecting fluid and became moribund in 96 hours so that these conditions may have been factors.

The question of insulin-epinephrine antagonism has been covered elsewhere (10). In interpreting the results of our experiments there is no reason for assuming such a gland correlation. In addition it was found previously (15) that the constant administration of epinephrine had no effect on the morphology of the islets of Langerhans and did not change the granule content of the alpha or beta cells.

The apparent effect of the presence or absence of sodium chloride in the injecting fluid is worthy of notice. The fact that the group of dogs not receiving sodium chloride became moribund after relatively short periods of epinephrine and glucose administration and showed on the average a more pronounced hyperglycemia and glycosuria is strongly suggestive of a disturbance in the acid-base equilibrium. This phase should be investigated more thoroughly. As a possible clinical application it may be that when glucose in 10 and 20 per cent solutions is intended for prolonged

intravenous administration it should be dissolved in a 0.3 to 0.6 per cent sodium chloride solution.

SUMMARY

1. Thirteen experiments were performed on unrestrained dogs in which a continuous intravenous injection was made: *a*, of epinephrine and glucose simultaneously; *b*, of epinephrine and glucose alternately. The length of the experiments was from 72 to 218 hours. The *rates* of administration were: for epinephrine, 0.060 to 0.120 mgm. per kgm. per hour; for glucose, 0.3 to 0.6 gram per kgm. per hour.

2. The continuous injection of epinephrine and glucose led to a marked hyperglycemia and glycosuria. Normal blood sugar values were obtained in from 36 to 48 hours and the glycosuria had disappeared by the end of 24 hours. With continued injection of epinephrine at even double the original rate there was never any further hyperglycemia or glycosuria. When the injection of epinephrine and glucose was stopped while the glycemia was within normal limits there was an abrupt fall in blood sugar values. On again administering epinephrine and glucose after a period of 24 hours or longer without injection, a hyperglycemia and glycosuria equalling or exceeding the first was obtained. When glucose alone was injected for at least 24 hours following the continuous administration of epinephrine alone, a second period of epinephrine injection led to a hyperglycemia and glycosuria which was a duplicate of the original.

3. The continuous injection of epinephrine and glucose had no definite effect in either increasing or decreasing the urinary total nitrogen excretion.

4. The effect of continuous injection on the total blood cholesterol was inconclusive. In the majority of experiments there appeared to be a slowly developing and transient increase in blood cholesterol values.

5. The possibility is suggested that the tissues contain reserve glycogen which can be mobilized by epinephrine. The amount can be estimated by the degree of glycosuria during the period of injection. The glycogen reserves remain depleted during the continuous administration of epinephrine and glucose and are not replenished until after epinephrine has ceased to act. When glucose is supplied in sufficient amounts the glycogen reserves may become larger than before, and the hyperglycemia and glycosuria during a second period of injection may be greater than the original. When no glucose is injected that available is insufficient to replenish the glycogen reserves in 24 hours and the hyperglycemia and glycosuria during a second period of epinephrine injection under these conditions are not as great as before.

The results of the present experiments are hard to interpret on the basis of an increased liver glycogen deposition under the influence of epinephrine injection. They do not offer evidence of increased insulin

secretion following epinephrine administration nor of suppression of carbohydrate metabolism.

Comment is made upon the apparent toxic effect of the absence of sodium chloride in the injecting fluid.

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